AD_____

Award Number: DAMD17-99-1-9072

TITLE: Role of the SOS Response in Stationary-Phase Hypermutation: A Model for Mutation in Oncogenesis and Chemotherapeutic Drug-Resistance

PRINCIPAL INVESTIGATOR: Gregory McKenzie

CONTRACTING ORGANIZATION:

Baylor College of Medicine
Houston, Texas 77030

REPORT DATE: July 2002

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Annual Summary (1 Jul 99 - 31 Jul 02)

4. TITLE AND SUBTITLE
Role of the SOS Response in Stationary-Phase Hypermutation: A Model for Mutation in Oncogenesis and Chemotherapeutic Drug-Resistance

5. FUNDING NUMBERS
DAMD17-99-1-9072

6. AUTHOR(S)
Gregory McKenzie

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
Houston, Texas 77030

Email: gm692056@bcm.tmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The SOS response of *Escherichia coli* is the prototypic DNA damage repair and cell cycle control system, analogous to checkpoint control in eukaryotes. The SOS response includes blocking the cell cycle, global mutagenesis via more than one mechanism, and up regulation of DNA repair and recombination functions. Adaptive mutation is a mutational program in non-growing cells subjected to starvation and so is also a temporary mutagenic response to environmental stress. It entails global hypermutation, and previously, the signal transduction pathway from the environment to the DNA was unknown. Previously, we demonstrated that adaptive mutation of a *lac* allele in *E. coli* is under control of the SOS response, and that SOS induced levels of component(s) other than or in addition to RecA (hRad51 homologue) are also involved. We have demonstrated that the SOS inducible error-prone polymerase DinB/DNA pol IV is required for most adaptive mutation. DNA pol IV is not required for growth-dependent mutation (indicating that it is specific to adaptive mutation) although genes of the *dinB* operon are. We have demonstrated the existence of this operon, and have evidence suggesting DNA pol IV is not required for recombination-primed DNA synthesis.

14. SUBJECT TERMS
breast cancer, DNA, SOS response

15. NUMBER OF PAGES
48

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

My work focuses on the role of the SOS response in Stressful Lifestyle Associated Mutation (SLAM). SLAM has a number of features that distinguish it from growth-dependent mutation including that: (i) SLAM occurs in the absence of growth during starvation; (ii) SLAM occurs in a hypermutable subset of the starving population; (iii) SLAM has a unique sequence spectrum of -1 deletions at mononucleotide repeats whereas growth-dependent Lac^+ reversions are heterogeneous; (iv) SLAM occurs in cells in which mismatch repair is limiting; and (v) SLAM requires homologous recombination proteins RecA, RecBC and RuvABC. The assay system that we use to study SLAM consists of *Escherichia coli* with a deletion of the *lac* region on the chromosome, and a *lacIΩZ* fusion carried on an F' conjugative plasmid. The *lac* fusion has a +1 frameshift in it, such that the cells are unable to grow on lactose (Lac^-). These cells are plated on medium containing lactose as the sole carbon source, and Lac^+ mutants appear every day, the vast majority of which are attributable to SLAM.

Body

During the last 3 years I have examined the role of the SOS response in SLAM. The SOS response is a DNA damage response in *E. coli* that induces expression of at least 42 genes involved in DNA repair and recombination, cell division inhibition, and induced mutagenesis. Expression of the regulon is normally repressed by the LexA repressor protein. Induction of the SOS response is thought to occur via sensing of damaged DNA (in the form of single-stranded DNA (ssDNA), an intermediate in most routes of DNA repair) by its interaction with RecA, a key recombination protein. Binding of ssDNA changes the conformation of RecA, activating a latent co-protease activity which promotes auto-cleavage of several targets, including the LexA repressor, phage λ CI repressor and the UmuD translesion synthesis protein. LexA cleavage de-represses the SOS regulon with the overall effect of promoting repair and recombination.

I have shown previously that efficient SLAM requires a functional SOS response because it requires LexA cleavage (McKenzie *et al.*, 2000). This indicates that induction of a Lex-repressed gene(s) is required for full levels of SLAM. One possible LexA-repressed candidate was the error-prone DNA polymerase IV (pol IV).

I examined the role of DNA pol IV in SLAM, and found that it was required for SLAM (and not for growth-dependent mutation) (McKenzie et al., 2001). This disagreed with data from another laboratory who showed that DNA pol IV was required for growth-dependent mutation. I have since shown that the gene encoding DNA pol IV (*dinB*) is transcribed with at least 2 other open reading frames, and one of these open-reading frames is required for growth-dependent mutation (McKenzie et al., 2002). This manuscript is in preparation for publication.

Due to the known requirements for double-strand break-repair recombination proteins, and other unpublished data from our laboratory, we postulated that SLAM takes place as part of DNA double-strand break-repair. Specifically that during SLAM double-strand break-repair recombination is directly associated with error-prone DNA synthesis by

DNA pol IV. This suggests further that DNA pol IV may be required for (or involved in) normal replicational recombination, which accounts for half of all double-strand break-repair recombination where its been measured. I spent several months testing this hypothesis using a phage λ recombination system, and gathered evidence that DNA pol IV is not required for replicational recombination. It may be involved in this process, but it appears to not be essential.

I have also supervised the work of many students in the Rosenberg laboratory. During the last 3 years, I have been responsible for training 2 undergraduates, and 7 graduate students on their laboratory rotations.

Key accomplishments (July 1999-July 2002)

- demonstrated LexA regulon involvement in SLAM (McKenzie et al., 2000)
- demonstrated that the chromosome of *E. coli* has similar requirements for mutation as F' plasmid (Bull et al., 2000)
- demonstrated that DNA polymerase IV is required for SLAM (McKenzie et al., 2001)
- wrote a review of SLAM and hypermutation in pathogenic bacteria for *Current Opinion in Microbiology* (McKenzie and Rosenberg, 2001)
- demonstrated *dinB* (encoding DNA pol IV) is transcribed in an operon with at least 2 other open reading frames (McKenzie et al., 2002) and that one of those open reading frames is required for growth-dependent mutation
- mentoring 7 graduate and 2 undergraduate students in mutation and recombination projects

Reportable outcomes (July 2000-July 2001)

Platform presentations:

2002. Harold M. Weintraub Graduate Student Award Symposium given by Fred Hutchinson Cancer Center

2000. Molecular & Human Genetics Department Retreat. Galveston, TX.

2000. Lost Pines Molecular Biology Conference. Lost Pines, Texas. Biological function of *E. coli* DinB/DNA polymerase IV in adaptive mutation.

2000. Molecular Genetics of Bacteria and Phages Meeting. Cold Spring Harbor Laboratory. Biological function of *E. coli* DinB/DNA polymerase IV in adaptive mutation.

1999. Molecular Genetics of Bacteria and Phages Meeting. University of Wisconsin, Madison. The SOS response in stationary-phase mutation.

Poster presentations:

2001. Molecular Genetics of Bacteria and Phages Meeting. University of Wisconsin, Madison. Inducible, mutagenic DNA polymerase IV (DinB) in recombination-dependent adaptive mutation.

2001. Genetic Recombination and Chromosome Rearrangements (FASEB Summer Conference). Snowmass, CO. Inducible, mutagenic DNA polymerase IV (DinB) in recombination-dependent adaptive mutation.

1999. Molecular & Human Genetics Department Retreat. Columbia Lakes, TX.

Conclusions:

In my doctoral thesis I have shown that adaptive mutation in the *E. coli lac* frameshift system is regulated by the SOS response to DNA damage via the LexA repressor. In particular, adaptive mutation requires a properly controlled SOS response for full levels of mutation, because both inhibiting and de-repressing an SOS response inhibits adaptive mutation. Further, I have shown that the inhibition conferred by a fully de-repressed LexA regulon is caused by the F'-encoded protein PsiB. The SOS response during adaptive mutation is likely to proceed *via* the *recF*-dependent pathway of SOS-induction, rather than the *recBC*-dependent pathway, because *recF* and *lexA3(Ind⁻)* mutations are epistatic.

I have demonstrated that the SOS-induced error-prone polymerase DNA polymerase IV is responsible for most *lac* adaptive mutation, but not growth-dependent *lac* mutation. I have shown that DNA pol IV is able to promote mutation at a variety of mononucleotide repeats, and that DNA pol III and DNA pol IV may account for almost all adaptive mutation. I have shown that DNA pol IV is not required for survival of insult by ultraviolet radiation or oxidative damage. I have examined the possibility that DNA pol IV is required for replicational recombination, and have evidence suggesting it is not.

I have discovered that the *dinB* gene is the first gene in an operon of at least three and perhaps four LexA-regulated SOS-induced genes, *dinB-yafN-yafO-(yafP)*. I have clarified why one group saw a growth-dependent mutation phenotype for a DNA pol IV mutant and we did not (McKenzie et al., 2001). The *dinB* insertion-deletion allele they used disrupts expression of the downstream genes, and loss of one or more of those genes is responsible for the phenotype of the insertion-deletion allele of *dinB*.

This work greatly expands our knowledge of the role of the SOS response and the error-prone DNA polymerase IV in mutation in starving, stressed cells, and in the *lac* frameshift system in particular. It supports the intriguing idea that the error-prone polymerases, like DNA pol IV of *E. coli*, that are found in all organisms may have the generation of mutation, not simply DNA repair, as part of their function.

References:

Bull, H. J., McKenzie, G. J., Hastings, P. J., and Rosenberg, S. M. (2000). Evidence that stationary-phase hypermutation in the *Escherichia coli* chromosome is promoted by recombination. Genetics *154*, 1427-1437.

McKenzie, G. J., Harris, R. S., Lee, P. L., and Rosenberg, S. M. (2000). The SOS response regulates adaptive mutation. Proc Natl Acad Sci USA *97*, 6646-6651.

McKenzie, G. J., Lee, P. L., Lombardo, M.-J., Hastings, P. J., and Rosenberg, S. M. (2001). SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Mol Cell *7*, 571-579.

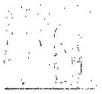
McKenzie, G. J., Lee, P. L., and Rosenberg, S. M. (2002). The SOS-regulated *dinB* operon and spontaneous mutation in *E. coli*. in preparation.

McKenzie, G. J., and Rosenberg, S. M. (2001). Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens. Curr Opin Microbiol *4*, 586-594.

reprints from

Current Opinion in

Microbiology



Elsevier Science London
84 Theobald's Road
London
UK WC1X 8RR
Tel +44 (0)20 7611 4000
Fax +44 (0)20 7611 4001

Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens

Gregory J McKenzie* and Susan M Rosenberg*†‡§

'Adaptive' or 'stationary-phase' mutation is a collection of stress responses promoting mutations, some of which are advantageous. In 2000 and 2001, in *Escherichia coli*, adaptive gene amplification was documented, and a parallel adaptive point-mutation mechanism was linked to the error-prone DNA polymerase, DinB (pol IV). We suggest that DinB homologues may contribute to adaptive strategies of pathogens, including antigenic variation.

Addresses
*Departments of Molecular and Human Genetics, †Biochemistry, and ‡Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Room S809 Mail Stop 225, Houston, Texas 77030-3411, USA
§e-mail: smr@bcm.tmc.edu

Current Opinion in Microbiology 2001, 4:586–594

1369-5274/01/$ – see front matter

Abbreviations

DSB	double-strand break
DSBR	double-stranded-break repair
DSE	double-strand end
MMR	mismatch repair
pol	DNA polymerase
VSG	variant surface glycoprotein

Introduction

'Stationary-phase' mutation denotes a collection of stress responses in which cells exposed to non-lethal stresses respond by promoting mutations. Some stationary-phase mutations may confer an advantage in the growth-limiting environment, and so are called 'adaptive' mutations, whereas others confer no known special advantage. The existence of stress-promoted mutation mechanisms implies that evolution may be hastened during stress (reviewed in [1••]). Stationary-phase mutations have been reported in several different bacterial and yeast assay systems, under various kinds of stress, and occurring by various mutation mechanisms including transposon-mediated insertions and deletions, substitution and frameshift (i.e. point) mutations and gene amplification. Thus, there is no universal stationary-phase mutation mechanism, but rather, at least a few distinct ones that may be specific to the particular kinds of environmental stress applied, or the genotypes of the cells assayed, or both. Here, we review recent advances from studies of a *lac* frameshift reversion assay system in *Escherichia coli* [2] in which a recombination-protein-dependent stationary-phase mutation mechanism occurs (see [1••] for a recent comprehensive review). These advances include the documentation of adaptive gene amplification [3••]; the demonstration that chromosomal (not just plasmid-borne) genes can undergo recombination-dependent mutation [4••]; and the discovery that a special error-prone DNA polymerase, DinB (pol IV) is required for adaptive point mutation in this system [5••]. (See [1••,6•] for reviews of this and other adaptive mutation mechanisms.) The Lac system represents a mechanism of inducible genetic change under stress that uses homologous recombination proteins and a special mutator DNA polymerase. We consider programmed genetic change responses in prokaryotic and eukaryotic pathogens and suggest that some of these may work similarly, using mutator DNA polymerases of the DinB/UmuC superfamily.

Stationary-phase point mutation in the Lac system

In the Lac frameshift reversion assay [2], *E. coli* cells whose chromosomal *lac* (lactose catabolism) genes have been deleted and that harbor an F′ conjugative plasmid carrying a *lac* +1 frameshift allele are spread onto solid lactose minimal medium, on which they cannot grow. Any Lac$^+$ mutants formed during growth of the cultures before plating on lactose appear as colonies in about two days. Additional Lac$^+$ colonies accumulate over the next week, and result from stationary-phase mutation mechanisms that occur after exposure to the lactose medium (reviewed in [1••]) by two distinct mechanisms, one producing point mutations and the other producing gene amplifications.

Recombination-dependent stationary-phase point mutation

Most of the late (stationary-phase or adaptive) Lac$^+$ mutant colonies carry frameshift reversions, nearly all of which are –1 deletions in small mononucleotide repeats [7,8]. In contrast, growth-dependent Lac$^+$ mutations are more heterogeneous [7,8]. Simple repeat deletions resemble DNA polymerase errors formed by a template slippage mechanism (reviewed in [1••]). Such errors are usually corrected by the post-replicative mismatch repair (MMR) system. However, MMR becomes limiting during stationary-phase mutation in this system, at the level of limiting MutL protein ([9,10]; see [11•,12•] for further discussion). The mechanism of stationary-phase-specific MMR limitation in this system is not yet understood. Because the number of MutL molecules per cell does not decline during lactose starvation, two (non-exclusive) possibilities seem reasonable [10]. MutL levels might decline only in cells generating mutations (which, as discussed below, appear to be a small subpopulation of cells), or MutL might be titrated by excess polymerase errors, or both. The stationary-phase mutation mechanism requires homologous recombination and double-strand-break repair (DSBR) proteins RecA, RecBC and RuvABC [13–15], implicating both DNA double-strand

breaks (DSBs) or double-strand ends (DSEs) and recombination in the process, either directly or indirectly [1••]. In direct models (Figure 1), recombinational repair of DSEs (formed in stationary phase by any number of possible mechanisms [1••]) is proposed to prime DNA replication, during which polymerase errors occur, leading to mutation at sites of DSBR [13–16]. Indirect models are also possible in which DSBR and mutation are not linked physically [1••]. An SOS response is required for efficient point mutation in the Lac system [2,17••]. SOS is the bacterial DNA damage repair and cell cycle checkpoint control response (reviewed in [18••]). The SOS response leads to the induction of *trans*-acting proteins involved in recombination, repair and mutation, including the error-prone DNA polymerase DinB (pol IV), which is required for most stationary-phase point mutation in this system [5••].

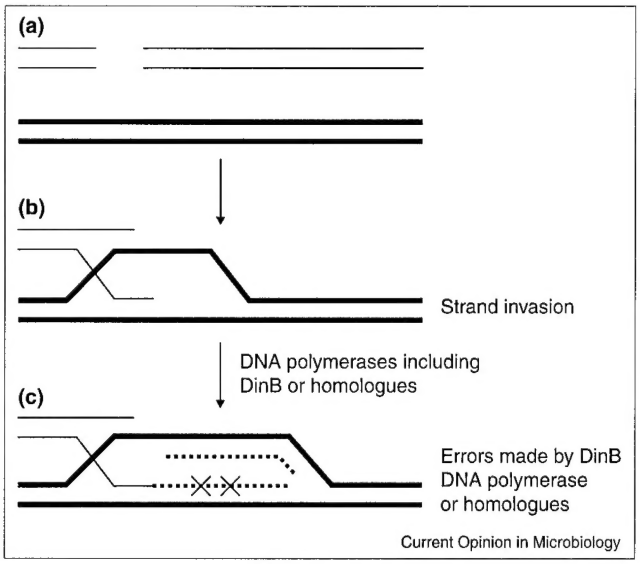
Cell subpopulations

Some or all of the point mutations in this system occur in a hypermutable subpopulation of cells (hypothesized by Hall [19]), as inferred from the high frequency of unselected mutations in other genes among Lac$^+$ revertants, but not among similarly starved Lac$^-$ cells [20,21••,22••]. See [1••,4••,21••,23•–25•] for discussion of whether one or more cell populations contributes to stationary-phase point mutation in the Lac system. These data (and others [4••,26]) also demonstrate that mutations in this system are not directed preferentially to *lac* or genes near it, as was hypothesized (reviewed in [1••]). The proposal that the selective environment induces transient hypermutation in a cell subpopulation has important implications for microbial populations under various stresses, including populations occupying the various niches that a pathogenic organism must pass through to colonize a host.

Recombination-dependent mutation in the bacterial chromosome

A long-standing issue in the Lac system is whether or not the distinct, recombination-protein-dependent mutation mechanism operating at *lac* on the F′ plasmid is also a mechanism of general genetic change for the bacterial chromosome. On the one hand, stationary-phase Lac$^+$ mutation on the F′ plasmid requires the transfer (Tra) functions of the F conjugative plasmid, although not actual DNA transfer [27–29]. Also, one *E. coli* [28] and one *Salmonella* [27] chromosomal site did not undergo RecA-dependent mutation in stationary phase in F$^-$ cells. Involvement of *trans*-acting plasmid-encoded functions has been suggested [22••]. On the other hand, hypermutation of chromosomal sites [20,21••,22••,23•] occurs during Lac$^+$ stationary-phase mutation, and does so with an uneven, hot and cold site distribution, as follows: one gene (*upp*) acquires 10 times more loss-of-function mutations than the entire maltose (Mal) or xylose (Xyl) fermentation regulons (>7 genes for Mal) [20], demonstrating that some sites or regions are more active for mutation ('hotter') than others. A key question is whether or not those chromosomal mutations occur via a mechanism similar to the one generating Lac$^+$ mutations on the F′ plasmid. Recent work shows that they do. Measuring frameshift reversion in a chromosomal tetracycline-resistance (*tet*) gene engineered into the chromosomal *upp* site in cells carrying the F′ plasmid, Bull *et al.* [4••] find that chromosomal TetR mutations increased during exposure to lactose medium in a RecA- and RuvC-dependent manner. The SOS mutator DNA polymerase, DinB, required specifically for stationary-phase mutation at *lac* [5••], is also required for chromosomal TetR mutation [4••]. Thus, recombination-protein- and DinB-dependent mutation is not limited to plasmid-borne sites. Whether or not *trans*-acting functions of the F′ plasmid are required has not been determined. Because most wild bacteria carry conjugative plasmids (and about 15% of *E. coli* and *Salmonella* carry F-homologous plasmids [30,31]), this stationary-phase mutation mechanism is likely to pertain to many different bacteria, regardless of whether or not conjugative plasmid functions are found to be required for mutation at chromosomal sites.

Figure 1

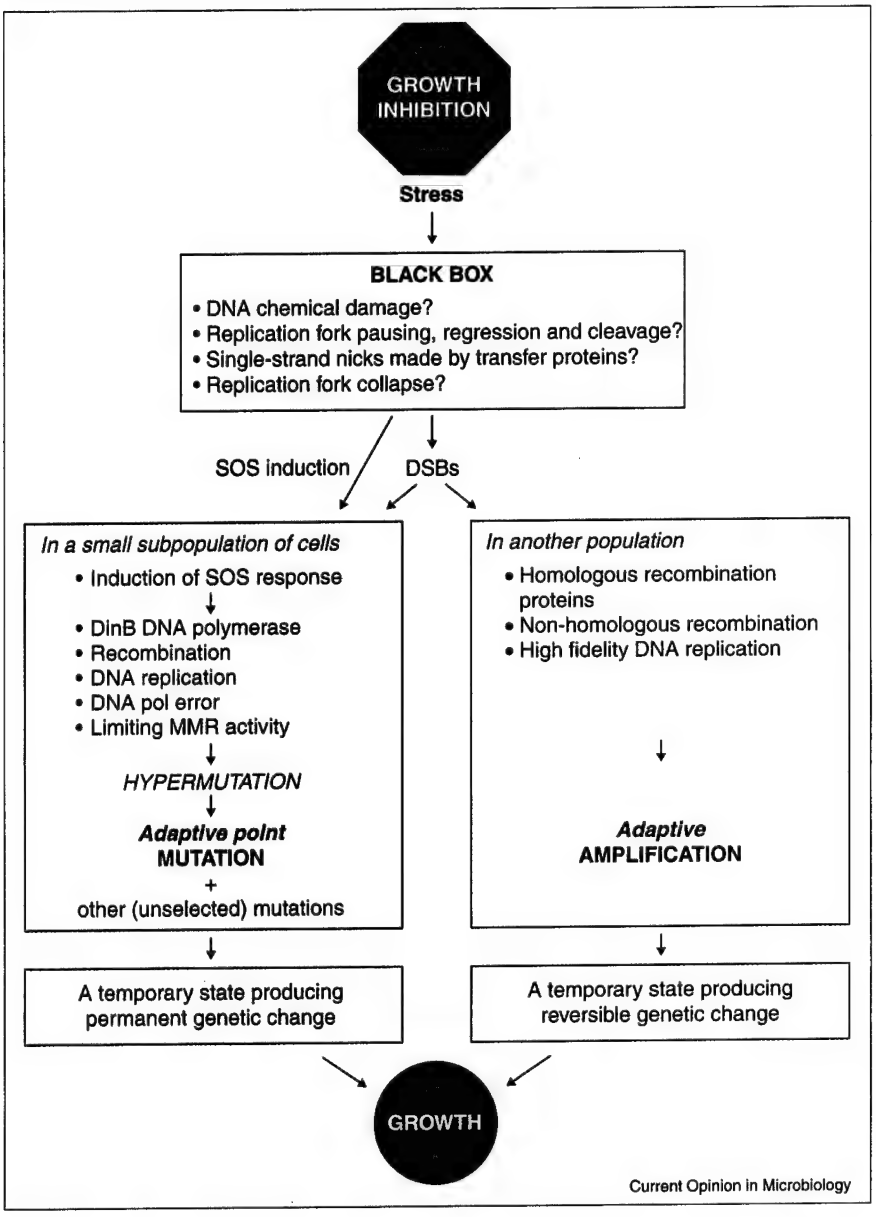


A model for both stationary-phase mutation in the *E. coli* Lac system and mutational antigenic variation, as seen in *T. brucei*. *T. brucei* has at least three homologues in the DinB/UmuC/Rad30/Rev1 family (Table 1). **(a)** A DNA double-strand break (DSB) in one molecule (thin lines) will be repaired by recombination with a homologous DNA region or molecule (thick lines). **(b)** Strand invasion of a homologous region of DNA primes **(c)** DNA synthesis (dotted lines). Errors made in this region by error-prone DNA polymerases persist as mutations. Possible sources of homology in stationary-phase bacteria are sister molecules, gene duplications, and DNA taken up from the environment [1••].

Adaptive amplification

In the year 2000, a second mechanism of stationary-phase genomic change was identified using the Lac assay [3••]. The *lac* +1 frameshift allele produces a small amount of β-galactosidase (1–2% of that of the wild-type gene). Amplification of this allele to 30–50 copies produces enough β-galactosidase to allow growth without acquisition of a compensatory frameshift mutation. Amplification was

Figure 2



Model for mechanisms of recombination-dependent adaptive point mutation and amplification in the *E. coli lac* frameshift reversion assay system. See text and [1••] for a review of data leading to this scheme. During the stress of starvation, DSBs or DSEs are proposed to be generated in the F′ plasmid and chromosomal DNA by any of several possible mechanisms [1••], summarized in the 'black box' (which indicates that the mechanism of DSB or DSE formation is not known). The point mutation response (blue text) includes induction of the SOS response to DNA damage and requires the SOS-controlled, error-prone DinB DNA polymerase, DNA recombination and replication functions, and produces polymerase errors that persist as mutations in an environment of limiting MMR activity. Figure 1 illustrates one model for this point mutation mechanism. The point mutation mechanism is also associated with hypermutation of unselected genes in the Lac$^+$ adaptive mutant cells. This is a transient mutable state that produces permanent genetic changes (point mutations), some of which allow the cells to grow. For adaptive amplification (purple text), the amplified DNA is present as direct repeats, the unique junctions of which have been mapped to regions of non-homologous joints [3••], as observed previously in bacterial amplification (see references in [3••]). This suggests at least one initial non-homologous recombination event [1••,3••], although the dependence of total late Lac$^+$ colonies on homologous recombination proteins suggests Rec protein involvement in the adaptive amplification response as well. Perhaps Rec proteins process DSEs that engage in non-homologous recombination leading to amplification [1••]. Amplification does not require an SOS response or DinB DNA polymerase [5••] and the amplified isolates are not hypermutated as Lac$^+$ point mutants are [3••]. Thus, the two appear to arise from different subpopulations of the starving cells. The adaptive amplification response produces reversible genetic changes (the direct repeats of amplified DNA can return to single copy by recombination) that allow growth.

shown to be adaptive, that is, formed in response to the lactose (selective) medium [3••], and is a reversible genetic change that allows escape from the stress of starvation. Amplification had been suggested to be an intermediate leading to point mutation in the Lac system [16]. However, the recent study shows that amplification and point mutation are parallel pathways — amplified DNA does not lead readily to point mutation in this system [3••]. The amplification and point mutation pathways are distinguished further; whereas adaptive point mutation requires an SOS response [2,17••] and SOS-controlled DinB [5••], adaptive amplification requires neither [5••]. Figure 2 illustrates a scheme for the mechanisms of the parallel adaptive point mutation and amplification mechanisms in the Lac system.

Mutator DNA polymerases of the DinB/UmuC superfamily

The study of mutation has been energized by the discovery that many organisms encode error-prone DNA polymerases of the newly found DinB/UmuC/Rad30/Rev1 superfamily (reviewed in [32••,33••]). Polymerases of this superfamily are found in prokaryotes, eukaryotes and archaea. Knowledge of this superfamily's existence has increased the number of DNA polymerases known in *E. coli* from three to

five, and has added four new DNA polymerases to those known in humans, prompting questions about their function(s). Some of these polymerases make errors more than 100 times more frequently than normal replicative polymerases do [34••,35••,36•–38•,39••,40•,41•]. Many function in DNA damage tolerance or repair. For example, the human tumor suppressor protein XPV (encoded by *RAD30A*) [32••,33••] and $UmuD_2'C$ (pol V) of *E. coli* [35••,39••] are translesion DNA polymerases. These polymerases insert bases opposite to sites of DNA base damage that otherwise blocks replication, and so allow damage tolerance when repair is incomplete. Most of these polymerases examined *in vitro* make errors on lesion-containing and undamaged DNA templates. Many are thought to make mutations *in vivo* as misincorporation errors in translesion synthesis opposite damaged bases or abasic sites [42••]. However, not all of these polymerases have known lesion bypass activity. The error-prone nature of these polymerases has led to proposals [32••,43•] of roles in mutational processes under cellular control, such as somatic hypermutation within immunoglobulin genes, in which two DinB/UmuC superfamily polymerases and also the *REV3*-encoded error-prone polymerase have now been implicated (reviewed in [44••]).

DinB/pol IV and its role in mutation

The *dinB* gene, encoding pol IV in *E. coli*, was discovered in a screen for *d*amage-*in*ducible (*din*) genes that are upregulated as part of the SOS response [45]. The gene was cloned later under the name *dinP*, and although the *dinB* designation has precedence [46], *dinP* is used commonly in sequence annotation of genomes. Phenotypes associated with *dinB* mutations or overexpression suggest a role in mutation in undamaged DNA. First, cells carrying an insertion in *dinB* are defective in phage-λ-untargeted mutagenesis (reviewed in [18••,32••,33••]), in which phage λ infecting *E. coli* cells irradiated with UV light experience tenfold to 100-fold higher mutation frequencies than do phages infecting non-irradiated hosts. Because the phage DNA itself is not irradiated, this suggests that *E. coli* pol IV increases mutation in undamaged DNA. Second, overproduction of *E. coli* pol IV *in vivo* leads to a fourfold to 800-fold increase in mutation in the absence of DNA-damaging agents [46,47•]. Both substitution and frameshift mutations are elevated, with frameshifts at mononucleotide repeats increased 100-fold to 800-fold. Purified *E. coli* pol IV is an error-prone DNA polymerase [34••] that makes both frameshift mutations and substitutions on undamaged DNA templates. It is not capable of translesion synthesis across typical damaged bases *in vitro*. Thus, it is possible that *E. coli* pol IV is not a translesion polymerase, and that mutations attributed to it *in vivo* may result from synthesis on undamaged template DNA. Work done *in vivo* apparently contradicting this idea is difficult to interpret: *dinB* appears to be the first gene in a putative *E. coli* operon containing four genes (see [5••] and references therein). Studies suggesting a loss of translesion mutation *in vivo* in cells deleted for *dinB* [48•] and part of the next gene downstream (see [5••]) are thus not yet definitive regarding a role for *E. coli* pol IV in mutation opposite lesions.

What function does DinB serve in *E. coli*? Recent work on stationary-phase mutation in the Lac system indicates that one of its functions is in promoting mutations in the *E. coli* genome under stress. DinB is required for recombination-dependent stationary-phase mutation both at *lac* on the F′ [5••], and at the chromosomal *upp–tet* site of Bull *et al.* [4••]. DinB is required specifically for mutation in stationary phase, and not in growing cells (see [5••], for reference to and discussion of an apparently contradictory report). These results, generated with a non-polar *dinB* allele, allow unambiguous assignment of a role for *E. coli* pol IV in stress-inducible mutation. By extension, other members of the DinB family that are present in other organisms, and whose functions are not yet known, may play similar roles. Two other DNA polymerases are induced during an SOS response in *E. coli*: the well-characterized error-prone lesion bypass polymerase pol V ($UmuD_2'C$) and the high-fidelity polymerase pol II. Neither of these is required for stationary-phase mutation in the Lac system ([2,17••]; references in [1••]).

In the 1970s and 1980s, Radman [49] and Echols [50] suggested that the SOS response might include inducible mutation enzymes, hastening evolution during dire circumstances in which genetic stasis is disadvantageous. Both *E. coli* pol V and pol IV may play such roles. Mutation promotion may be an important function of these enzymes, regardless of whether or not these polymerases also function in DNA damage tolerance or repair, which, after all, become necessary during stress. DinB may be a mutation enzyme, working to generate mutation in undamaged DNA or at a type of endogenous damage yet to be determined.

Antigenic variation

Antigenic variation refers to a collection of processes by which pathogenic microbes change their surface antigens to avoid detection by the host immune response (reviewed in [51]). Surface antigens subject to antigenic variation include porins, pili, fimbriae and other surface molecules. Antigenic variation mechanisms fall into several broad categories: recombinational, mutational and transcriptional mechanisms.

Trypanosoma brucei

The eukaryotic pathogen *Trypanosoma brucei* appears to use all three mechanisms of antigenic variation (reviewed in [52,53]) for the expression of variant surface glycoproteins (VSGs). *T. brucei* belongs to the family of African trypanosomes that cause sleeping sickness, and contains about 1000 VSG genes in its genome, of which only one is expressed at a time [54]. The change from expression of one VSG to another occurs at variable rates, between 10^{-2} and 10^{-6} per cell per generation (see references in [51]).

Table 1

Some pathogenic (and other) microbes that carry DinB/UmuC superfamily homologues.

Major taxonomic division	Genus
Prokaryotes	
Firmicutes	
Bacillaceae	*Bacillus**†
	Staphylococcus
	Mycoplasma
	Ureaplasma
Clostridiaceae	*Clostridium*‡§
	*Enterococcus**
	Lactococcus#
	*Streptococcus**†§
	Corynebacterium§
	*Mycobacterium**
Proteobacteria	
α subdivision	*Caulobacter*
	Mesorhizobium¶
	Sinorhizobium¥
β subdivision	*Bordetella*§
	Burkholderia§
	Neisseria†
γ subdivision	*Actinobacillus*†
	Escherichia
	*Klebsiella***
	Legionella††
	Pasteurella
	*Pseudomonas**
	Salmonella§**‡‡
	*Shewanella**
	Vibrio
	Yersinia§
	*Geobacter**
	*Desulfovibrio**
Spirochaetales	*Treponema* *§§ (but not *T. pallidum*)
Green non-sulphur bacteria	*Dehalococcoides**
Eukaryotes	*Candida*§
	Saccharomyces
	Schizosaccharomyces§
	*Plasmodium**§¥
	*Trypanosoma**
Archaea	*Halobacterium*##
	Sulfolobus

Table 1 legend

This table summarises the results of a non-exhaustive BLAST search [79] for *dinB* homologues in some pathogens and other microbes. One or more species of the genera listed possess sequences with at least 25% sequence identity or 42% sequence similarity to the *E. coli dinB* gene. This search does not discriminate between branches of the DinB/UmuDC/Rad30/Rev1 superfamily of DNA polymerases. A more detailed summary of the results of this search, including references to the published sequence data used, is posted at http://www.imgen.bcm.tmc.edu/rosenberg/mchDinB-UmuC_table.html. Unpublished preliminary sequence data were obtained from sequences deposited in the NCBI Unfinished Genomes website by the following organizations: *The Institute for Genomic Research, URL http://www.tigr.org; †The University of Oklahoma's Advanced Center for Genome Technology, URL http://www.genome.ou.edu; ‡The Genome Therapeutics Corporation, URL http://www.cric.com; §The Sanger Centre, URL http://www.sanger.ac.uk; #GENOSCOPE, URL http://www.genoscope.cnrs.fr/; ¶Kazusa DNA Research Institute, URL http://www.kazusa.or.jp/en/; ¥Stanford Genome Technology Center, URL http://www-sequence.stanford.edu; **Genome Sequencing Center in Washington University in St Louis, URL: http://genome.wustl.edu/gsc/; ††Columbia Genome Center, URL http://genome3.cpmc.columbia.edu/~legion/; ‡‡University of Illinois Urbana Champaign, URL http://www.salmonella.org/; §§University of Texas Health Sciences Center, URL http://www-mmg.med.uth.tmc.edu/sphaeroides/; ##Institute for Systems Biology, URL http://www.systemsbiology.org

VSG expression is thought to occur from only one of about 20 telomere-linked sites in the genome [55,56]. The remainder are transcriptionally silenced [57], resulting in a system in which VSG expression can be accomplished in a number of ways. A silent VSG copy can be recombined into an expression site [58], or altered transcription patterns in the cell can lead to VSG transcripts from alternative telomere-linked copies [57].

Mutation-mediated antigenic variation in this system is apparent from inspection of recombinants after silent VSGs are moved into the transcriptionally active site. As many as one nucleotide in 100 are mutated in the newly recombined VSG (these changes are not present in the silent copy) [58,59]. The expressed copy of a VSG appears to be mutated during recombination into the transcriptionally active site, generating new epitopes without jeopardizing the parent gene from the genome.

There are some interesting commonalities between antigenic variation in *T. brucei* and stationary-phase mutation in the *E. coli* Lac system. Stationary-phase mutation requires the homologous recombination protein RecA [2,13], and VSG antigenic variation requires the eukaryotic RecA homologue Rad51 [60]. *E. coli* stationary-phase mutation occurs more frequently at some (hot) sites than other (cold) sites (reviewed in [1••]) and, in *T. brucei*, mutations appear to occur only within the open reading frame (ORF) of the newly expressed VSG [58]. This led to the postulate that VSG mutation occurs via an RNA intermediate and sloppy reverse transcriptases expressed from one of the many retrotransposons found in *T. brucei*. We propose an alternative model in which homologous recombination primes DNA synthesis involving an error-prone DNA polymerase, and these errors persist as mutations (as shown in Figure 1). In support of this model and its proposed similarity to the Lac$^+$ point mutation mechanism, *T. brucei* has at least three homologues in the DinB/UmuC/Rad30/Rev1 superfamily (a DinB, a Rad30 and a Rev1 homologue; see Table 1).

Prokaryotes

Mutation plays a slightly different role in antigenic variation in prokaryotes. Many prokaryotic pathogens use mutation as a regulatory tool to turn on and off expression of various different surface protein genes [61]. Typically, these genes have a simple nucleotide-repeat tract within the promoter region (in transcriptional control), or in early regions of the ORF (in translational control). Changes in the length of the tract result in the promoter being on or off (for transcriptional mechanisms), or result in either shortened or full-length protein being produced (for translational mechanisms). For example, in *Mycoplasma fermentans*, transcription of P78, which is part of an ATP-binding

cassette (ABC) transporter, requires the presence of a tract of seven adenine nucleotides in the mRNA [62]. Deletion of a single adenine nucleotides results in loss of expression of P78, and loss of that particular surface antigen.

Bacterial genera that use strand-slippage regulatory mechanisms include *Bordetella* [63], *Campylobacter* [64], *Haemophilus* [65], *Mycoplasma* [62,66] and *Neisseria* [67]. Antigenic variation in these cases typically occurs at rates of 10^{-2}–10^{-5} per cell per generation. The mechanism of these antigenic variation events is largely unexplored, but Moxon *et al.* [61] suggest that these are regulated. We suggest that DinB and its homologues are candidates for involvement in mutational antigenic variation. Regulation of antigenic variation could be accomplished by increased expression of DinB during times of stress. Stress (for example, oxidative stress) could be caused in the context of an immune response. If this were the case, then the resulting mutations would be adaptive in the same sense that Lac$^+$ mutations are adaptive in the *E. coli* system. In BLAST searches of partly completed microbial genomes, we find that most prokaryotes carry homologues of the DinB/UmuC/Rad30/Rev1 superfamily, including all of the genera (except *Haemophilus*) listed above (Table 1). For example, *Bordetella* and *Neisseria* carry homologues of DinB that have at least 45% sequence identity and 63% sequence similarity to DinB. This model predicts that mutations affecting *dinB* homologues will prevent or decrease mutational antigenic variation.

Mutation in pathogens in general

In several systems, heritable mutator mutants (notably, bacterial cells defective in MMR, with mutation rates 10–100 times higher than that of wild-type cells) make up a small proportion of the population of bacterial cells in a chronic infection [68–70,71••]. This suggests that a high mutation rate is beneficial, providing new adaptations to the changing, stressful environment of a host. However, less than 10% of the population of bacterial cells in an infected host are mutator mutants. This suggests either that the benefit of being a mutator is a transient one, and regaining a wild-type MMR gene is required for long-term success of a population [72••,73••,74•,75••], or that many cells in these populations undergo periods of transiently high mutation rate without heritable loss of repair protein genes (reviewed in [1••]). We prefer the idea that both transient and heritable mutator states contribute to the long-term survivability and evolvability of microbial species. Mutability may be a characteristic selected for in pathogens as they pass through severe bottlenecks in population size and must generate diversity *de novo* each time they infect a host [74•]. We suggest that induction of mutator DNA polymerases could produce a transient mutator state both directly by excess errors and also indirectly by errors titrating MMR [10,5••], thereby producing transient MMR-deficiency without loss of MMR genes. This might account for the many successfully adapted pathogens that have not lost MMR genes. Transient mutability would be a survival mechanism without the long-term costs of mutability suffered after adaptation to the stress [75••].

Antibiotic resistance also contributes to pathogenesis and can be acquired by mutational mechanisms [76••] that might be inducible by stressful environments including those selecting resistance [77,78••]. Even lethal antibiotics cause non-lethal stress at lower concentrations that must occur frequently in patients and in nature [76••]. Transient hypermutation like that occurring in the Lac system has been suggested as a basis for multiple drug resistance in *Mycobacterium* [78••].

Conclusions

So far, the only roles demonstrated conclusively for the prototype of the DinB family, pol IV of *E. coli*, are in the induction of mutation on apparently undamaged DNA. Whether or not DinB also functions in DNA damage repair or tolerance, the 'mutator' aspect of its function, leads us to propose that it and its homologues might be important in circumstances in which mutations are beneficial. In microbial pathogens, such circumstances could include antigentic variation, antibiotic resistance and generally hastened evolution via transient mutator induction by titration of MMR proteins. During these circumstances, mutability may be a programmed response, as it appears to be in stationary-phase mutation. Investigation of phenotypes of cells lacking DinB homologues may support this hypothesis. We look forward to better understanding of the functions of DinB homologues in microbial pathogens.

Acknowledgements

We apologize to many colleagues whose important papers could not be cited owing to severe space limitations. We thank TA Cebula for discussion, J Davies, PJ Hastings, D Low, ER Moxon, J Petrosino and AL Smith for comments on the manuscript, and M-J Lombardo for editorial assistance. The organizations listed in Table 1 contributed sequence data prior to publication. GJM is supported by a Department of Defense Breast Cancer Fellowship. Supported by grants R01-GM53158 and R01-AI43917 from the National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Rosenberg SM: **Evolving responsively: adaptive mutation.** *Nat Rev Genet* 2001, **2**:504-515.

A recent comprehensive review of adaptive mutation in the *E. coli* Lac system and other bacterial and yeast adaptive mutation mechanisms. Rigorous experimental definitions of 'adaptiveness' are discussed, and an indirect model for the role of recombination proteins in stationary-phase mutations is considered, as are functions of eukaryotic DinB/UmuC superfamily polymerases and programmed mutation in eukaryotes.

2. Cairns J, Foster PL: **Adaptive reversion of a frameshift mutation in *Escherichia coli*.** *Genetics* 1991, **128**:695-701.

3. •• Hastings PJ, Bull HJ, Klump JR, Rosenberg SM: **Adaptive amplification. An inducible chromosomal instability mechanism.** *Cell* 2000, **103**:723-731.

Rigorous evidence is presented that shows that gene amplification in *E. coli* can be an adaptive response, induced by conditions that select the amplified DNA. Also, a model in which adaptive Lac$^+$ mutations were postulated to be standard growth-dependent mutations accumulated in multiple copies of amplified DNA [16] is tested and fails tests of three of its predictions. Amplification does not appear to be a precursor to adaptive Lac$^+$ point mutation in the *E. coli* system.

4. Bull HJ, Lombardo M-J, Rosenberg SM: **Stationary-phase mutation •• in the bacterial chromosome: recombination protein and DNA polymerase IV dependence.** *Proc Natl Acad Sci USA* 2001, **98**:8334-8341.

The controversial issue of whether or not the recombination-dependent mechanism of stationary-phase mutation described in studies of F′-located genes applies to chromosomal sites is addressed with the first report of recombination protein- and pol-IV-dependent mutation at a site in the *E. coli* chromosome. See also [23•].

5. McKenzie GJ, Lee PL, Lombardo M-J, Hastings PJ, Rosenberg SM: •• **SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification.** *Mol Cell* 2001, **7**:571-579.

This paper demonstrates that DinB (pol IV), of the DinB/UmuC polymerase superfamily, is required for stationary-phase and not growth-dependent mutation in the Lac system. A non-polar *dinB* mutation is used. This paper also reviews previous work in which growth-dependent frameshift mutation was thought to be pol IV-dependent. Adaptive amplification is distinguished further from point mutation by the fact that it does not require an SOS response or pol IV.

6. Foster PL: **Mechanisms of stationary phase mutation: a decade of • adaptive mutation.** *Annu Rev Genet* 1999, **33**:57-88.

A review of several bacterial adaptive mutation assay systems and mechanisms. The conclusions regarding lack of involvement of the SOS response in Lac stationary-phase mutation have been superceded (since [17••]). This paper provides an excellent reference list to many bacterial studies.

7. Rosenberg SM, Longerich S, Gee P, Harris RS: **Adaptive mutation by deletions in small mononucleotide repeats.** *Science* 1994, **265**:405-407.

8. Foster PL, Trimarchi JM: **Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs.** *Science* 1994, **265**:407-409.

9. Longerich S, Galloway AM, Harris RS, Wong C, Rosenberg SM: **Adaptive mutation sequences reproduced by mismatch repair deficiency.** *Proc Natl Acad Sci USA* 1995, **92**:12017-12020.

10. Harris RS, Feng G, Ross KJ, Sidhu R, Thulin C, Longerich S, Szigety SK, Winkler ME, Rosenberg SM: **Mismatch repair protein MutL becomes limiting during stationary-phase mutation.** *Genes Dev* 1997, **11**:2426-2437.

11. Foster PL: **Are adaptive mutations due to a decline in mismatch • repair? The evidence is lacking.** *Mutat Res* 1999, **436**:179-184.

A criticism of the interpretations of the results in [10], in which overproduction of MutL is shown to inhibit stationary-phase but not growth-dependent mutation in the Lac system. The interpretation [10] is that MutL becomes limiting for MMR activity specifically during stationary-phase mutation, and several possible mechanisms for this are presented, some of which are explored further in [1••,5••].

12. Harris RS, Feng G, Ross KJ, Sidhu R, Thulin C, Longerich S, Szigety SK, • Hastings PJ, Winkler ME, Rosenberg SM: **Mismatch repair is diminished during stationary-phase mutation.** *Mutat Res* 1999, **437**:51-60.

A rebuttal to the criticism of [11•]. Data are presented that demonstrate the need for control experiments argued for in [10] and against in [11•].

13. Harris RS, Longerich S, Rosenberg SM: **Recombination in adaptive mutation.** *Science* 1994, **264**:258-260.

14. Harris RS, Ross KJ, Rosenberg SM: **Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation.** *Genetics* 1996, **142**:681-691.

15. Foster PL, Trimarchi JM, Maurer RA: **Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*.** *Genetics* 1996, **142**:25-37.

16. Andersson DI, Slechta ES, Roth JR: **Evidence that gene amplification underlies adaptive mutability of the bacterial *lac* operon.** *Science* 1998, **282**:1133-1135.

17. McKenzie GJ, Harris RS, Lee PL, Rosenberg SM: **The SOS response •• regulates adaptive mutation.** *Proc Natl Acad Sci USA* 2000, **97**:6646-6651.

This paper reports that an SOS response is required for Lac$^+$ stationary-phase mutation (as also shown in [2]), including a requirement for induction of LexA-controlled function(s) other than RecA. A partial requirement for RecF and the discovery that F-encoded PsiB is an inhibitor of Lac stationary-phase mutation are reported.

18. Sutton MD, Smith BT, Godoy VG, Walker GC: **The SOS •• response: recent insights into *umuDC*-dependent mutagenesis and DNA damage tolerance.** *Annu Rev Genet* 2000, **34**:479-497.

An excellent recent review of the SOS response and the two DinB/UmuC superfamily DNA polymerases under its control: UmuD′C (pol V) and DinB (pol IV).

19. Hall BG: **Spontaneous point mutations that occur more often when advantageous than when neutral.** *Genetics* 1990, **126**:5-16.

20. Torkelson J, Harris RS, Lombardo M-J, Nagendran J, Thulin C, Rosenberg SM: **Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation.** *EMBO J* 1997, **16**:3303-3311.

21. Rosche WA, Foster PL: **The role of transient hypermutators in •• adaptive mutation in *Escherichia coli*.** *Proc Natl Acad Sci USA* 1999, **96**:6862-6867.

This paper confirms previous findings that Lac$^+$ adaptive revertants are hypermutated at unselected genes [20] and interprets the similar findings differently: in terms of there being two mutable populations (rather than one hypermutable population). This idea is discussed further in [1••,4••,23•,24•,25•].

22. Godoy VG, Gizatullin FS, Fox MS: **Some features of the mutability •• of bacteria during nonlethal selection.** *Genetics* 2000, **154**:49-59.

Previous findings that Lac$^+$ adaptive revertants are hypermutated at unselected genes [20] are confirmed, and the possibility that the F′ contributes *trans*-acting promoters of stationary-phase mutation is suggested. A study of a set of different F′ plasmids and their effects on stationary-phase mutation must be viewed cautiously because of the lack of isogenicity of the plasmids used.

23. Bull HJ, McKenzie GJ, Hastings PJ, Rosenberg SM: **Evidence that • stationary-phase hypermutation in the *Escherichia coli* chromosome is promoted by recombination.** *Genetics* 2000, **154**:1427-1437.

This paper presents evidence that suggests that unselected mutation associated with adaptive Lac reversion is also recombination-promoted: *recD* and *recG* mutations, which cause hyper-adaptive Lac reversion, are shown to increase chromosomal mutations associated with Lac$^+$. This supports the idea that chromosomal secondary mutations form via a mechanism similar to most adaptive Lac reversion (demonstrated subsequently [4••]), and in doing so, discourages the idea that Lac$^+$ mutants carrying secondary mutations represent a minority mutation pathway (suggested in [21••,24•]).

24. Cairns J: **The contribution of bacterial hypermutators to mutation • in stationary phase.** *Genetics* 2000, **156**:923.

The author argues that most Lac$^+$ do not arise from a hypermutable cell population.

25. Bull HJ, McKenzie GJ, Hastings PJ, Rosenberg SM: **The contribution • of transiently hypermutable cells to mutation in stationary phase.** *Genetics* 2000, **156**:925-926.

The authors respond to Cairns' letter [24•] with the caution that the data supporting the two-population model are not statistically significant (see also [1••]), and that the mechanistic similarity between Lac$^+$ and associated secondary mutations supports a common origin.

26. Foster PL: **Nonadaptive mutations occur in the F′ episome during adaptive mutation conditions in *Escherichia coli*.** *J Bacteriol* 1997, **179**:1550-1554.

27. Galitski T, Roth JR: **Evidence that F′ transfer replication underlies apparent adaptive mutation.** *Science* 1995, **268**:421-423.

28. Foster PL, Trimarchi JM: **Adaptive reversion of an episomal frameshift mutation in *Escherichia coli* requires conjugal functions but not actual conjugation.** *Proc Natl Acad Sci USA* 1995, **92**:5487-5490.

29. Foster PL, Trimarchi JM: **Conjugation is not required for adaptive reversion of an episomal frameshift mutation in *Escherichia coli*.** *J Bacteriol* 1995, **177**:6670-6671.

30. Boyd EF, Hartl DL: **Recent horizontal transmission of plasmids between natural populations of *Escherichia coli* and *Salmonella enterica*.** *J Bacteriol* 1997, **179**:1622-1627.

31. Boyd EF, Hill CW, Rich SM, Hartl DL: **Mosaic structure of plasmids from natural populations of *Escherichia coli*.** *Genetics* 1996, **143**:1091-1100.

32. Gerlach VL, Aravind L, Gotway G, Schultz RA, Koonin EV, •• Friedberg EC: **Human and mouse homologs of *Escherichia coli* DinB (DNA polymerase IV), members of the UmuC/DinB superfamily.** *Proc Natl Acad Sci USA* 1999, **96**:11922-11927.

An excellent review of the DinB/UmuC polymerase superfamily that also reports the tissue-specific localization of mouse DinB homologue, DinB1.

33. Goodman MF, Tippin B: **Sloppier copier DNA polymerases •• involved in genome repair.** *Curr Opin Genet Dev* 2000, **10**:162-168.

An excellent review of the new error-prone DNA polymerases of the DinB/UmuC superfamily, especially the *E. coli* SOS UV-lesion bypass polymerase, UmuD′C (pol V).

34. Wagner J, Gruz P, Kim SR, Yamada M, Matsui K, Fuchs RP, Nohmi T:
•• **The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis.** *Mol Cell* 1999, **4**:281-286.
The authors of this paper demonstrate that the *dinB* gene product is an error-prone DNA polymerase.

35. Tang M, Shen X, Frank EG, O'Donnell M, Woodgate R, Goodman MF:
•• **UmuD'(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V.** *Proc Natl Acad Sci USA* 1999, **96**:8919-8924.
The authors demonstrate that the *E. coli* SOS-controlled UV-mutagenesis and lesion bypass function, UmuD'C, is an error-prone DNA polymerase. This led to the recognition that the vast number of *umuC* homologous genes represented in all three domains of life constitute a new DNA polymerase superfamily.

36. Johnson RE, Prakash S, Prakash L: **The human DINB1 gene**
• **encodes the DNA polymerase Pol theta.** *Proc Natl Acad Sci USA* 2000, **97**:3838-3843.
The authors of this paper demonstrate that human DinB homologue, DINB1, is a DNA polymerase, called pol theta here, pol kappa by many other authors.

37. Ohashi E, Ogi T, Kusumoto R, Iwai S, Masutani C, Hanaoka F,
• Ohmori H: **Error-prone bypass of certain DNA lesions by the human DNA polymerase kappa.** *Genes Dev* 2000, **14**:1589-1594.
This paper demonstrates the ability of human DinB homologue, DINB1, to insert bases across form abasic sites and N-2-acetyleaminefluorine (AAF), but not opposite thymine dimers or cisplatin adducts.

38. Ohashi E, Bebenek K, Matsuda T, Feaver WJ, Gerlach VL,
• Friedberg EC, Ohmori H, Kunkel TA: **Fidelity and processivity of DNA synthesis by DNA polymerase kappa, the product of the human *DINB1* gene.** *J Biol Chem* 2000, **275**:39678-39684.
Characterization of error-prone translesion synthesis by the human DinB homologue, DINB1.

39. Tang M, Pham P, Shen X, Taylor JS, O'Donnell M, Woodgate R,
•• Goodman MF: **Roles of *E. coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis.** *Nature* 2000, **404**:1014-1018.
The authors of this paper report the biochemical characterization of the error-prone polymerases pol IV and pol V, showing translesion synthesis activity of the latter but not the former.

40. Zhang Y, Yuan F, Xin H, Wu X, Rajpal DK, Yang D, Wang Z: **Human**
• **DNA polymerase kappa synthesizes DNA with extraordinarily low fidelity.** *Nucleic Acids Res* 2000, **28**:4147-4156.
A quantitative demonstration that DINB1 is a low-fidelity enzyme (on undamaged template DNA) that incorporates one wrong nucleotide per 200 nucleotides incorporated.

41. Gerlach VL, Feaver WJ, Fischhaber PL, Friedberg EC: **Purification**
• **and characterization of pol kappa, a DNA polymerase encoded by the human *DINB1* gene.** *J Biol Chem* 2001, **276**:92-98.
A more complete characterization of DINB1 that shows that it lacks a proofreading activity and is not stimulated by the DNA polymerase sliding clamp, PCNA (unlike DinB, which is more processive when the *E. coli* sliding clamp is added).

42. Pham P, Bertram JG, O'Donnell M, Woodgate R, Goodman MF:
•• **A model for SOS-lesion-targeted mutations in *Escherichia coli.*** *Nature* 2001, **409**:366-370.
The authors of this paper present a detailed model for the molecular mechanism of UmuD'C-mediated lesion bypass replication.

43. Ogi T, Kato T, Ohmori H: **Mutation enhancement by DINB1, a**
• **mammalian homologue of the *Escherichia coli* mutagenesis protein DinB.** *Genes Cells* 1999, **4**:607-618.
This paper presents evidence that human DINB1 is a mutation-promoting enzyme, like its bacterial homologue, DinB.

44. Storb U: **DNA polymerases in immunity: profiting from errors.** *Nat*
•• *Immunol* 2001, **2**:484-485.
The author of this paper reviews three recent papers that implicate, via various methods, the *REV3*-encoded error-prone polymerase zeta (not of the DinB/UmuC superfamily) and also DinB/UmuC superfamily polymerases DINB1 and RAD30A in somatic hypermutation.

45. Kenyon CJ, Walker GC: **DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli.*** *Proc Natl Acad Sci USA* 1980, **77**:2819-2823.

46. Kim SR, Maenhaut-Michel G, Yamada M, Yamamoto Y, Matsui K, Sofuni T, Nohmi T, Ohmori H: **Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an overexpression of *dinB/dinP* results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA.** *Proc Natl Acad Sci USA* 1997, **94**:13792-13797.

47. Wagner J, Nohmi T: ***Escherichia coli* DNA polymerase IV mutator**
• **activity: genetic requirements and mutational specificity.** *J Bacteriol* 2000, **182**:4587-4595.
This paper corroborates and extends the evidence of [46], showing that overproduction of pol IV causes mutations *in vivo*. A forward mutation assay is used, in which frameshift and substitution mutations are seen to be promoted. Also, evidence suggesting that excess errors produced by pol IV could titrate MMR is presented.

48. Napolitano R, Janel-Bintz R, Wagner J, Fuchs RP: **All three SOS**
• **inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis.** *EMBO J* 2000, **19**:6259-6265.
This paper reports that mutation opposite benzo(*a*)pyrene base adducts require *E. coli* pol V and pol IV. Use of a large, polar *dinB* deletion, removing part of the next gene in its operon, necessitates use of caution in interpreting a role for pol IV.

49. Radman M: **SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis.** *Basic Life Sci* 1975, **5A**:355-367.

50. Echols H: **SOS functions, cancer and inducible evolution.** *Cell* 1981, **25**:1-2.

51. Deitsch KW, Moxon ER, Wellems TE: **Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections.** *Microbiol Mol Biol Rev* 1997, **61**:281-293.

52. Donelson JE, Hill KL, El-Sayed NM: **Multiple mechanisms of immune evasion by African trypanosomes.** *Mol Biochem Parasitol* 1998, **91**:51-66.

53. Cross GA, Wirtz LE, Navarro M: **Regulation of *vsg* expression site transcription and switching in *Trypanosoma brucei.*** *Mol Biochem Parasitol* 1998, **91**:77-91.

54. Van der Ploeg LH, Valerio D, De Lange T, Bernards A, Borst P, Grosveld FG: **An analysis of cosmid clones of nuclear DNA from *Trypanosoma brucei* shows that the genes for variant surface glycoproteins are clustered in the genome.** *Nucleic Acids Res* 1982, **10**:5905-5923.

55. Myler PJ, Allison J, Agabian N, Stuart K: **Antigenic variation in African trypanosomes by gene replacement or activation of alternate telomeres.** *Cell* 1984, **39**:203-211.

56. Milhausen M, Nelson RG, Parsons M, Newport G, Stuart K, Agabian N: **Molecular characterization of initial variants from the IsTat I serodeme of *Trypanosoma brucei.*** *Mol Biochem Parasitol* 1983, **9**:241-254.

57. Vanhamme L, Poelvoorde P, Pays A, Tebabi P, Van Xong H, Pays E: **Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei.*** *Mol Microbiol* 2000, **36**:328-340.

58. Lu Y, Hall T, Gay LS, Donelson JE: **Point mutations are associated with a gene duplication leading to the bloodstream reexpression of a trypanosome metacyclic VSG.** *Cell* 1993, **72**:397-406.

59. Lu Y, Alarcon CM, Hall T, Reddy LV, Donelson JE: **A strand bias occurs in point mutations associated with variant surface glycoprotein gene conversion in *Trypanosoma rhodesiense.*** *Mol Cell Biol* 1994, **14**:3971-3980.

60. McCulloch R, Barry JD: **A role for RAD51 and homologous recombination in *Trypanosoma brucei* antigenic variation.** *Genes Dev* 1999, **13**:2875-2888.

61. Moxon ER, Rainey PB, Nowak MA, Lenski RE: **Adaptive evolution of highly mutable loci in pathogenic bacteria.** *Curr Biol* 1994, **4**:24-33.

62. Theiss P, Wise KS: **Localized frameshift mutation generates selective, high-frequency phase variation of a surface lipoprotein encoded by a mycoplasma ABC transporter operon.** *J Bacteriol* 1997, **179**:4013-4022.

63. Willems R, Paul A, van der Heide HG, ter Avest AR, Mooi FR: **Fimbrial phase variation in *Bordetella pertussis*: a novel mechanism for transcriptional regulation.** *EMBO J* 1990, **9**:2803-2809.

64. Park SF, Purdy D, Leach S: **Localized reversible frameshift mutation in the *flhA* gene confers phase variability to flagellin gene expression in *Campylobacter coli.*** *J Bacteriol* 2000, **182**:207-210.

65. Weiser JN, Love JM, Moxon ER: **The molecular mechanism of phase variation of *H. influenzae* lipopolysaccharide.** *Cell* 1989, **59**:657-665.

66. Zhang Q, Wise KS: **Localized reversible frameshift mutation in an adhesin gene confers a phase-variable adherence phenotype in mycoplasma.** *Mol Microbiol* 1997, **25**:859-869.

67. Stern A, Brown M, Nickel P, Meyer TF: **Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation.** *Cell* 1986, **47**:61-71.

68. LeClerc JE, Li B, Payne WL, Cebula TA: **High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens.** *Science* 1996, **274**:1208-1211.

69. Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E, Denamur E, Elion J: **Highly variable mutation rates in commensal and pathogenic *Escherichia coli*.** *Science* 1997, **277**:1833-1834.

70. Picard B, Duriez P, Gouriou S, Matic I, Denamur E, Taddei F: **Mutator natural *Escherichia coli* isolates have an unusual virulence phenotype.** *Infect Immun* 2001, **69**:9-14.

71. •• Oliver A, Cantón R, Campo P, Baquero F, Blázquez J: **High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection.** *Science* 2000, **288**:1251-1254.
The authors of this paper provide evidence for high frequencies of mutator mutants in antibiotic-resistant bacterial pathogens.

72. •• Denamur E, Lecointre G, Darlu P, Tenaillon O, Acquaviva C, Sayada C, Sunjevaric I, Rothstein R, Elion J, Taddei F *et al.*: **Evolutionary implications of the frequent horizontal transfer of mismatch repair genes.** *Cell* 2000, **103**:711-721.
Phylogenetic evidence for frequent loss and re-acquisition of MMR genes in bacterial evolution. A similar conclusion was reached by the authors of [73••].

73. •• Brown EW, LeClerc JE, Li B, Payne WL, Cebula TA: **Phylogenetic evidence for horizontal transfer of *mutS* alleles among naturally occurring *Escherichia coli* strains.** *J Bacteriol* 2001, **183**:1631-1644.
Phylogenetic evidence for frequent loss and re-acquisition of MMR genes in bacterial evolution. A similar conclusion was reached by the authors of [72••].

74. • Ochman H, Moran NA: **Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis.** *Science* 2001, **292**:1096-1099.
The authors of this paper review the evidence for loss and re-acquisition of genes in bacterial evolution.

75. •• Giraud A, Matic I, Tenaillon O, Clara A, Radman M, Fons M, Taddei F: **Cost and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut.** *Science* 2001, **291**:2606-2608.
The authors of this paper provide evidence that colonization of a new host by bacteria requires adaptive genetic changes, the formation of which is facilitated by being mutator. Additionally, once the adaptive mutations have been acquired, a non-mutator condition is favored.

76. •• Martinez JL, Baquero F: **Mutation frequencies and antibiotic resistance.** *Antimicrob Agents Chemother* 2000, **44**:1771-1777.
An excellent review of the role of mutation in acquisition of antibiotic resistance.

77. Riesenfeld C, Everett M, Piddock LJV, Hall BG: **Adaptive mutations produce resistance to ciprofloxacin.** *Antimicrob Agents Chemother* 1997, **41**:2059-2060.

78. •• Karunakaran P, Davies J: **Genetic antagonism and hypermutability in *Mycobacterium smegmatis*.** *J Bacteriol* 2000, **182**:3331-3335.
Evidence that many mutations are required for multiple antibiotic resistances: those that confer the resistance and others that ameliorate the negative effects of the resistance-conferring mutations on cell growth. The authors suggest that all of these may occur during a transient hypermutation akin to adaptive mutation in the Lac system.

79. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res* 1997, **25**:3389-3402.

Evidence That Stationary-Phase Hypermutation in the *Escherichia coli* Chromosome Is Promoted by Recombination

Harold J. Bull,*,† Gregory J. McKenzie,*,† P. J. Hastings* and Susan M. Rosenberg*,†

**Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030-3498 and*
†*Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada*

Manuscript received September 5, 1999
Accepted for publication December 10, 1999

ABSTRACT

Adaptive (or stationary-phase) mutation is a group of phenomena in which mutations appear to occur more often when selected than when not. They may represent cellular responses to the environment in which the genome is altered to allow survival. The best-characterized assay system and mechanism is reversion of a *lac* allele on an F′ sex plasmid in *Escherichia coli*, in which the stationary-phase mutability requires homologous recombination functions. A key issue has concerned whether the recombination-dependent mutation mechanism is F′ specific or is general. Hypermutation of chromosomal genes occurs in association with adaptive Lac$^+$ mutation. Here we present evidence that the chromosomal hypermutation is promoted by recombination. Hyperrecombinagenic *recD* cells show elevated chromosomal hypermutation. Further, *recG* mutation, which promotes accumulation of recombination intermediates proposed to prime replication and mutation, also stimulates chromosomal hypermutation. The coincident mutations at *lac* (on the F′) and chromosomal genes behave as independent events, whereas coincident mutations at *lac* and other F-linked sites do not. This implies that transient covalent linkage of F′ and chromosomal DNA (Hfr formation) does not underlie chromosomal mutation. The data suggest that recombinational stationary-phase mutation occurs in the bacterial chromosome and thus can be a general strategy for programmed genetic change.

STATIONARY-PHASE (or adaptive) mutations occur in nondividing or slowly growing cells exposed to a nonlethal selection (reviewed by Drake 1991; Foster 1993; Hall 1993; Symonds 1993; Rosenberg *et al.* 1994; Rosenberg 1997; Lombardo *et al.* 1999a; Lombardo and Rosenberg 1999). They differ from spontaneous growth-dependent mutations, which occur in dividing cells, before exposure to an environment selective for the mutation, and randomly in the genome (*e.g.*, Luria and Delbrück 1943). In some assay systems for stationary-phase mutation, the mutations may occur preferentially in genes whose functions are selected (Wright *et al.* 1999). In the system used here, genome-wide hypermutability appears to underlie adaptive mutations (*i.e.*, those mutations selected) and produce non-adaptive mutations concurrently (Torkelson *et al.* 1997; postulated by Hall 1990; Ninio 1991), although nonrandomness in the form of "hot" and "cold" sites for the mutation has been documented (Rosenberg 1997; Torkelson *et al.* 1997). Stationary-phase mutations form via multiple different mechanisms, some of which clearly differ from spontaneous growth-dependent mutation (Maenhaut-Michel and Shapiro 1994; Hall 1995; Maenhaut-Michel *et al.* 1997; Rosenberg 1997; Taddei *et al.* 1997; Wright *et al.* 1999). The molecular mechanisms of mutation in nongrowing and slowly growing cells under stress provide important models for evolution of microbes in real-world, stressful environments, for mutations that confer resistance to antibiotics and chemotherapeutic drugs, and for mutations that initiate cancer in cells that are not growing actively. Elucidation of mechanisms of mutation in response to selection is modifying core concepts in biological evolution and development (*e.g.*, Cairns *et al.* 1988; Culotta 1994; Thaler 1994; Shapiro 1997; Pennisi 1998; Caporale 1999). Understanding these mechanisms will illuminate their roles in evolution, development, cancer formation, and genome structure and function, all of which may be underpinned by such dynamic mutational processes.

The best-studied assay for stationary-phase mutation uses *Escherichia coli* cells carrying a revertible *lac* frameshift allele on an F′ sex plasmid and no *lac* genes in the chromosome (Cairns and Foster 1991). Growth-dependent Lac$^+$ revertants, carrying mutations formed prior to plating on lactose minimal medium, appear after about 2 days of incubation on lactose plates. Additional Lac$^+$ mutant colonies appear each day for several days and these carry mutations formed during starvation on the lactose medium (McKenzie *et al.* 1998; stationary-phase mutations). The stationary-phase mutations form via a unique molecular mechanism that differs from growth-dependent Lac$^+$ mutations as follows:

Corresponding author: Susan M. Rosenberg, Department of Molecular and Human Genetics, Baylor College of Medicine, 1 Baylor Plaza, Rm. S809A, Mail Stop BCM225, Houston, TX 77030-3498.
E-mail: smr@bcm.tmc.edu

1. Homologous recombination functions *recA*, *recB*, *ruvA*, *ruvB*, and *ruvC* are required for stationary-phase, but not growth-dependent Lac$^+$ mutation (Harris *et al.* 1994, 1996; Foster *et al.* 1996).
2. Because RecBCD loads onto DNA only at double-strand DNA ends (DSEs), DSEs are implicated as molecular intermediates in the mutagenic process (Harris *et al.* 1994).
3. Formation of stationary-phase Lac$^+$ mutations requires F-encoded transfer functions (Foster and Trimarchi 1995a; Galitski and Roth 1995), but not actual F plasmid transfer (Foster and Trimarchi 1995a,b; Radicella *et al.* 1995; Rosenberg *et al.* 1995). One possible explanation for this requirement is that the single-strand nick produced at the transfer origin by transfer (Tra) proteins develops into a double-strand break (DSB) and that this is the major DSB source on the F plasmid (Kuzminov 1995; Rosenberg *et al.* 1995).
4. Stationary-phase Lac$^+$ mutations are nearly all -1 deletions in small mononucleotide repeats, whereas the growth-dependent Lac$^+$ mutations are heterogeneous (Foster and Trimarchi 1994; Rosenberg *et al.* 1994).
5. The stationary-phase mutations are attributable to DNA polymerase errors made by the major replicative polymerase, PolIII (Foster *et al.* 1995; Harris *et al.* 1997a).
6. These errors persist under conditions of insufficient postreplicative mismatch repair (MMR) activity (Longerich *et al.* 1995), during which the MutL MMR protein becomes limiting (Harris *et al.* 1997b, 1999a).

The recombination-dependent stationary-phase mutations are proposed to result from DNA replication at sites of DSB repair via homologous recombination (Harris *et al.* 1994; reviewed by Rosenberg 1997; Lombardo and Rosenberg 1999) as follows: DSBs are suggested to occur during the stress of starvation on lactose medium (see Harris *et al.* 1994; Kuzminov 1995; Rosenberg *et al.* 1995, 1996; Bridges 1997; Seigneur *et al.* 1998, for suggestions on how DSBs could form). RecBCD loads onto DSEs and digests and unwinds the DNA, producing single-stranded DNA ends, which are used by RecA protein for strand invasion of a homologous DNA molecule (Figure 1). The D loops are proposed to prime DNA replication (Harris *et al.* 1994; Kogoma 1997; see Liu *et al.* 1999; Motamedi *et al.* 1999) using DNA PolIII (Foster *et al.* 1995; Harris *et al.* 1997a). Polymerase errors are suggested to persist due to transient MMR deficiency (Longerich *et al.* 1995; Harris *et al.* 1997b). These become Lac$^+$ (and other) mutations.

The "adaptive" nature of these mutations can be accounted for by a modification of Hall's proposal in which adaptive mutations arise in a hypermutable subpopulation of cells exposed to selection (Hall 1990; see Ninio 1991). Both nonadaptive and adaptive (Lac$^+$) mutations are proposed to form. However, the nonadaptive mutations might not be readily apparent in the main population either because of their low number or due to death of mutant cells that had not also acquired an adaptive mutation. This model was supported in the Lac system by the demonstrations of high frequencies of mutation at multiple sites, genome-wide, in Lac$^+$ colony formers, but not in the main population of (Lac$^-$) cells exposed to selection (Torkelson *et al.* 1997; Rosche

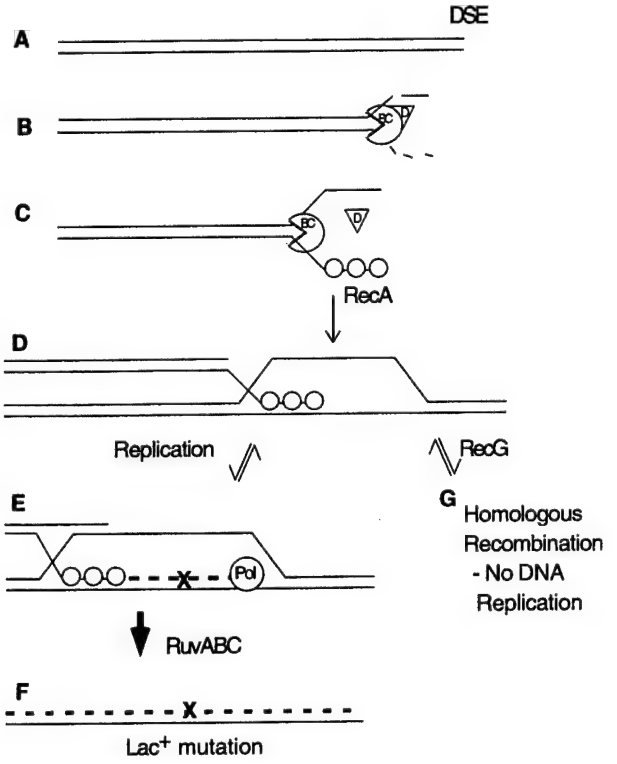


Figure 1.—A model for formation of recombination-dependent stationary-phase Lac$^+$ mutations. (A) A double-strand end (DSE) is proposed to occur [*e.g.*, via processing of a Tra-dependent nick (Rosenberg *et al.* 1995), disintegration (Kuzminov 1995) or stalling (Rosenberg *et al.* 1996; Michel *et al.* 1997; Seigneur *et al.* 1998) of a replication fork, or other mechanism (*e.g.*, Rosenberg 1994, 1997; Bridges 1997)]. (B) The DSE is processed by the RecBCD enzyme, creating single-stranded DNA ends (C) that become bound by RecA (small circles), which catalyzes invasion of a homologous duplex to produce a displacement loop (D). (E) The invading strand (in this example a 3′-ended single strand) serves as a primer and loading site for the replicative DNA polymerase PolIII (Liu *et al.* 1999). Errors produced by PolIII (X) may remain uncorrected due to a transient deficiency in methyl-directed mismatch repair (Longerich *et al.* 1995; Harris *et al.* 1997b). The error becomes genetically fixed giving a Lac$^+$ mutation (X in F). An alternate outcome of intermediate D is that strand invasion (perhaps from 5′-ended single-strand invasions, which cannot serve as a primer) leads to (G) homologous recombination with no associated DNA replication (Harris *et al.* 1996; Rosenberg and Motamedi 1999; Motamedi *et al.* 1999). (- - -) Newly synthesized DNA.

and Foster 1999). These unselected mutations appear to form concurrently with the Lac$^+$ adaptive mutations (not during growth of the Lac$^+$ colony) as seen by their representation in all cells (not sectors) of the Lac$^+$ mutant colonies.

Although it is clear that (1) a fundamentally different mutation mechanism generates the Lac$^+$ stationary-phase mutations, (2) the cells engaging in this mechanism are differentiated [transiently mismatch-repair deficient (Longerich *et al.* 1995; Harris *et al.* 1997b) and comprising a small hypermutable subpopulation (Torkelson *et al.* 1997)], and also (3) chromosomal genes are mutated concurrently (Torkelson *et al.* 1997; Rosche and Foster 1999), the possible relevance of the recombination-dependent stationary-phase mutation mechanism to mutation in the bacterial chromosome has been controversial (*e.g.*, Foster and Trimarchi 1995a,b; Galitski and Roth 1995, 1996; Radicella *et al.* 1995; Peters *et al.* 1996; Benson 1997, discussed below). The issue underlying this question is whether the recombinational stationary-phase mutation mechanism affects the bacterial genome in general.

Recombination is a hallmark of this novel mutation mechanism. Here, we test the role of recombination in hypermutation of chromosomal genes that occurs concurrently with adaptive Lac$^+$ reversion. We find that two recombination-altered alleles, both of which promote recombination-dependent stationary-phase mutation at *lac* (on the F′), also promote concurrent hypermutation of chromosomal genes. The data imply that recombination-dependent stationary-phase mutation is not strictly an F-plasmid-specific mechanism, but rather is a mechanism for genetic change at multiple sites throughout the genome. We observe that mutations at *lac* and chromosomal sites occur as independent events, supportive of the idea that these sites are not joined covalently (as an Hfr) at the time of mutation. In contrast, mutation of *lac* and another F′-borne site does not appear to be independent. The data support the idea that recombination-dependent stationary-phase mutation is a mechanism for genetic change at multiple sites throughout the genome and thus may be a general response to stress and a strategy for evolution.

MATERIALS AND METHODS

***E. coli* strains:** A strain unable to revert to Lac$^+$ was used to scavenge carbon sources other than lactose (Cairns and Foster 1991). All other strains are derived from FC40 (Cairns and Foster 1991), which carries a large chromosomal deletion of the *lac* operon and neighboring genes, and an F′ sex plasmid carrying genes in the *lac* and *proAB* region. The *lac* allele on the F′ has a translational fusion of *lacI* with *lacZ* and a +1 frameshift mutation in *lacI* which is polar on *lacZ*. The *recD* derivative is SMR582 carrying *recD1903::*Tn*10miniTet* (Harris *et al.* 1994). The *recG* derivative is RSH316 carrying *recG258::*Tn*10miniKan* (Harris *et al.* 1996).

Mutation assays: Assays for Lac$^+$ stationary-phase mutation were performed as described (Harris *et al.* 1996). Assays for unselected secondary mutations were performed by replica-plating Lac$^+$ colonies, obtained in the Lac$^+$ assay after 5 days of incubation, to various indicator and selective media as described by Torkelson *et al.* (1997). All presumptive secondary mutants were confirmed by streaking from the original Lac$^+$ colony (master colony) for single colonies on the appropriate indicator plate. The purity of Lac$^+$ colonies expressing fermentation mutations was determined by removing the master colonies with plugs of agar, suspending the cells in buffer, diluting, and spreading on minimal (M9 thiamine) lactose plates to obtain $\sim$ 100 Lac$^+$ colonies per plate. The resulting Lac$^+$ colonies were replica-plated to the appropriate MacConkey indicator medium and the numbers of fermentation-defective mutants and fermentation-competent colonies were determined. Typically, >80% of the secondary mutant colonies assayed were pure in that all Lac$^+$ colonies replica-plated were of the mutant phenotype. Mutations resulting in 5-fluorocytosine resistance (5FC^r) map to *codAB* or *upp* whereas mutations resulting in 5-fluorouracil resistance (5FU^r) map only to *upp* (Torkelson *et al.* 1997). *upp* mutations were not useful in this study because we observed that both the *recD* and *recG* mutations are able to suppress the 5FU^r and 5FC^r phenotypes of a large (>80%) portion of the *upp* mutations (data not shown) and so only 5FC^r 5FU^s mutants were included. (Reconstruction experiments with known *upp* and *codA* mutations demonstrated that *upp* mutations that were suppressed for 5FU^r by *recD* and *recG* were also suppressed for 5FC^r. Thus all 5FC^r 5FU^s are at *codAB*.) 5FC^r colonies were tested for purity as described above. Typically, >80% of 5FC^r mutants identified in this manner were pure.

Unselected mutations in Lac$^-$ starved cells were assayed as described (Torkelson *et al.* 1997). Plugs of agar were removed from between visible Lac$^+$ colonies each day and suspended in M9 buffer. Aliquots were spread on LBH and on MacConkey lactose plates and incubated. (This allowed detection of any Lac$^+$ colonies that were not yet visible and had been picked accidentally.) The resulting Lac$^-$ colonies (each derived from a Lac$^-$ cell starved on lactose) were screened for unselected mutations by replica-plating.

RESULTS

Strategy for measuring stationary-phase mutation in the bacterial chromosome: Chromosomal mutations coincident with Lac$^+$ stationary-phase mutation can be measured by replica-plating the Lac$^+$ stationary-phase mutant colonies to media selective for particular loss-of-function mutants or to color indicator media for fermentation-defective mutants (Torkelson *et al.* 1997). The hypermutation of chromosomal genes is observed in the Lac$^+$ mutants only and not in the neighboring Lac$^-$ cells, which were also starved on lactose and then rescued, grown into colonies, and replica-plated. Such "Lac$^-$ stressed cell colonies" display low chromosomal mutation frequencies indistinguishable, in replica-plating assays, from Lac$^-$ cells never exposed to selection (Torkelson *et al.* 1997; Rosche and Foster 1999, and below). Therefore, to score stationary-phase hypermutation of chromosomal genes, we obtained Lac$^+$ stationary-phase mutants to screen for the presence of additional mutations.

For three reasons, we infer that these additional chromosomal mutations occurred during transient, station-

ary-phase hypermutability and not during subsequent growth of the Lac$^+$ mutant cell into a colony: first, the Lac$^+$ colonies with additional mutations are mostly pure, not mixed (sectored), for the additional mutation, implying that the initial colony-forming cell carried the mutation (Torkelson *et al.* 1997; and shown again here, see materials and methods). Second, the Lac$^+$ mutants are not heritable mutator mutants (Longerich *et al.* 1995; Torkelson *et al.* 1997) and, third, they are not heritable stationary-phase mutator mutants (Rosenberg *et al.* 1998); thus they must have descended from a transiently mutable subpopulation. Lac$^-$ stressed cells, which show low frequencies of additional mutation (Torkelson *et al.* 1997; Rosche and Foster 1999, and below), make up the main population.

In recombination-defective strains, no Lac$^+$ stationary-phase mutants arise (Harris *et al.* 1994, 1996; Foster *et al.* 1996). Therefore we tested the role of recombination in chromosomal hypermutation using recombination-proficient cells with elevated stationary-phase Lac$^+$ mutation, *recD* and *recG* null mutants.

Rationale for use of *recD* and *recG* mutants: We tested whether two recombination gene defects that promote recombination-dependent stationary-phase mutation of *lac* on the F′ affect mutability of chromosomal genes in stationary phase. *recD* null alleles confer hyperrecombination (Chaudhury and Smith 1984; Amundsen *et al.* 1986; Biek and Cohen 1986; Thaler *et al.* 1989) and enhance stationary-phase mutation (Harris *et al.* 1994; Foster and Rosche 1999). Strains carrying *recG* null mutations are hypermutable in Lac$^+$ stationary-phase mutation (Foster *et al.* 1996; Harris *et al.* 1996), and several lines of evidence imply that RecG protein, which is a Holliday junction branch migration helicase (Whitby *et al.* 1994), interferes with those recombination intermediates that promote replication (Whitby *et al.* 1993; Al-Deib *et al.* 1996; Harris *et al.* 1996; McGlynn *et al.* 1997). Thus both *recD* and *recG* mutations increase numbers of the strand-exchange recombination intermediates thought to promote replication and both promote recombination-dependent stationary-phase mutation (see Figure 1).

***recD* and *recG* increase mutability of chromosomal genes in Lac$^+$ stationary-phase mutants:** Otherwise isogenic *rec*$^+$, *recD*, and *recG* strains were starved in parallel on lactose minimal medium. Following the fifth day of lactose selection, the Lac$^+$ colonies were replica-plated to appropriate indicator and selective media to reveal chromosomal loss-of-function mutants. Chromosomal mutations assayed were among those detected previously by Torkelson *et al.* (1997; and see materials and methods). The results of three separate experiments are presented in Table 1 and Figure 2. The *recD* null mutant showed approximately twice as many xylose (Xyl$^-$) and maltose (Mal$^-$) fermentation-defective mutations per Lac$^+$ colony as did *rec*$^+$ cells. The increased frequency of chromosomal mutation coincident with Lac$^+$ is similar to the increase in Lac$^+$ stationary-phase mutant frequency in the *recD* background (Figure 2). Because *recD* strains are hyperrecombinagenic (Chaudhury and Smith 1984; Amundsen *et al.* 1986; Biek and Cohen 1986; Thaler *et al.* 1989), these data suggest that the increased mutability of chromosomal loci and F-borne loci is due to recombination.

In the *recG* null strain, the frequency of Xyl$^-$ and Mal$^-$ mutations per Lac$^+$ mutant was, respectively, 4.6-fold and 6.0-fold higher than in the *rec*$^+$ control (Table 1 and Figure 2B). Also, fructose fermentation-defective (Fru$^-$) mutations, which previously (Torkelson *et al.* 1997) and here were so infrequent as to be undetectable in *rec*$^+$ cells, were detected in the *recG* strain (Table 1 and Figure 2B). Thus, loss of RecG increases the frequency of chromosomal mutations concurrent with Lac$^+$ stationary-phase mutation. The total increase (Xyl$^-$ plus Mal$^-$ plus Fru$^-$) is at least 5.7-fold (Table 1 and Figure 2B). Because RecG is a helicase that can unwind and abort recombination intermediates (Whitby *et al.* 1993; Al-Deib *et al.* 1996; Harris *et al.* 1996; McGlynn *et al.* 1997) and that inhibits recombination-dependent stationary-phase mutation (Foster *et al.* 1996; Harris *et al.* 1996), these data suggest that allowing recombination intermediates to enter a replication-promoting pathway in the RecG-deficient strain promotes chromosomal mutation, in agreement with the *recD* data (above).

recD and *recG* strains increase mutation at unselected chromosomal loci to an extent similar to their effect on Lac$^+$ colony formation (Figure 2). However, their effect on unselected mutations is smaller than that seen on Lac$^+$ colony formation. It may be that chromosomal genes cannot be mutated with the same efficiency as F′-borne genes, perhaps because of some sequence specificity of the mutation mechanism. The apparent difference in *recD* and *recG* could reflect varying susceptibility to mutagenesis for the chromosomal loci. We have observed hot and cold sites for unselected chromosomal mutation (Rosenberg 1997; Torkelson *et al.* 1997).

Increased mutation is limited to the hypermutable subpopulation: The increase in chromosomal mutations per Lac$^+$ stationary-phase mutant in *recD* and *recG* strains indicates that *recD* and *recG* loss increases mutability in cells that become Lac$^+$. We wished to know whether the elevated mutability is specific to the hypermutable subpopulation cells or whether loss of *recD* or *recG* increases the mutability of all cells exposed to starvation on lactose medium. Because of the large numbers of replica-plated colonies required to detect chromosomal mutations among Lac$^-$ starved cells [one to two orders of magnitude less frequent than among Lac$^+$ colonies, at 10^{-4} to 10^{-5} of the whole population (Torkelson *et al.* 1997)], we tested only the *recG* strain. Stationary-phase mutation is elevated so dramatically by *recG* that a *recG*-promoted increase in chromosomal mutability should be readily detectable even in the Lac$^-$ cells.

TABLE 1

Chromosomal mutations per Lac$^+$ adaptive mutant are increased in *recG* and *recD* cells

rec genotype	Mutant phenotype	No. of mutants among Lac$^+$ adaptive revertants (mutant colonies/Lac$^+$ colonies scored)[a]			Frequency of unselected chromosomal mutations/Lac$^+$ adaptive revertant[b]	
		Expt. 1	Expt. 2	Expt. 3	Mean ± SEM	
rec$^+$	Xyl$^-$	4/4080	2/4675	6/6253	7.9×10^{-4}	
	Mal$^-$	2/4080	0/4675	8/6253	$<6.6 \times 10^{-4}$	
	Fruc$^-$	0/4080	0/4675	0/6253	$<2.1 \times 10^{-4}$	
	Total[c]	6/4080	2/4675	14/6253	1.4×10^{-3}	(0.53×10^{-3})
recD	Xyl$^-$	5/3639	2/1038	4/8712	1.3×10^{-3}	
	Mal$^-$	6/3639	2/1038	15/8712	1.8×10^{-3}	
	Fruc$^-$	1/3639	0/1038	0/8712	$<4.5 \times 10^{-3}$	
	Total[c]	12/3639	4/1038	19/8712	3.1×10^{-3}	(0.49×10^{-3})
recG	Xyl$^-$	19/6427	14/3960	8/1834	3.6×10^{-3}	
	Mal$^-$	25/6427	19/3960	6/1834	4.0×10^{-3}	
	Fruc$^-$	2/6427	1/3960	1/1834	3.7×10^{-3}	
	Total[c]	46/6427	34/3960	15/1834	8.0×10^{-3}	(0.43×10^{-3})

[a] Three experiments were done with the three strains assayed in parallel.
[b] The mean of the frequencies for the three experiments (±1 SE).
[c] Total numbers for all phenotypes, each experiment.

To assay chromosomal mutations among Lac$^-$ stressed cells, those cells were recovered from between visible Lac$^+$ colonies after prolonged starvation and replated nonselectively to form colonies that were then replica-plated to screen for chromosomal mutants (see MATERIALS AND METHODS and TORKELSON *et al.* 1997). The data in Table 2 indicate that the low frequency of Mal$^-$ and Xyl$^-$ mutations per Lac$^-$ stressed cell colony [one to two orders of magnitude lower than per Lac$^+$ mutant (Table 2; also reported by TORKELSON *et al.* 1997)] is not increased detectably by the *recG* mutation. By contrast, Mal$^-$ and Xyl$^-$ mutations are increased per Lac$^+$ colony (Tables 1 and 2; Figure 2). These data imply that promotion of mutation by the absence of RecG is limited to the hypermutable subpopulation cells.

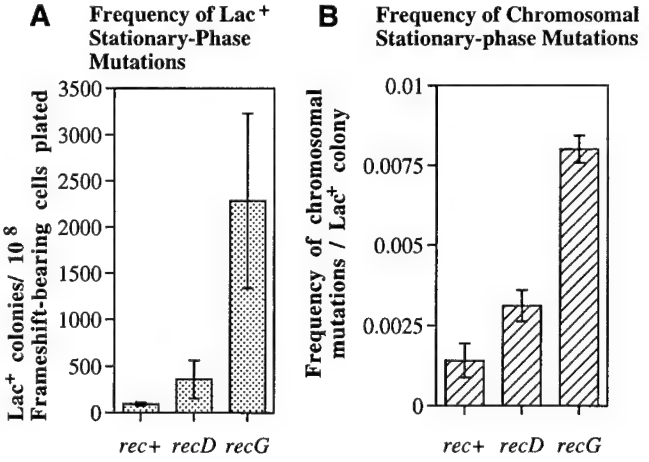


FIGURE 2.—(A) Lac$^+$ stationary-phase mutants accumulated over 5 days of selection in *rec*$^+$, *recD*, and *recG* strains. (B) The frequency of unselected chromosomal mutations (Mal$^-$, Xyl$^-$, and Fru$^-$) per Lac$^+$ stationary-phase mutant in *rec*$^+$, *recD*, and *recG* strains. The values are the mean of three separate experiments, with *rec*$^+$, *recD*, and *recG* tested in parallel each time [Table 1, total mean ± 1 SE (error bars)]. Values obtained for B are from the Lac$^+$ colonies reported in A.

Some condition present in the subpopulation, but not the main population, appears to be necessary for high levels of chromosomal stationary-phase mutation, as is the case for Lac$^+$ adaptive mutation. The condition that makes the subpopulation cells mutable could be the occurrence of DNA DSBs or DSEs at which recombination would occur (HARRIS *et al.* 1994), limiting MMR (HARRIS *et al.* 1997b), or other (TORKELSON *et al.* 1997; G. J. MCKENZIE, R. S. HARRIS, P. L. LEE and S. M. ROSENBERG, unpublished results).

Independent events underlie mutation of *lac* and chromosomal but not F′-linked genes: Previously, F′-linked as well as chromosomal genes were hypermutated in Lac$^+$ stationary-phase mutants (TORKELSON *et al.*

TABLE 2

***recG* increases mutation specifically in the Lac$^+$ population**

Genotype	Mutants/Lac$^+$ colony		Mutants/Lac$^-$ colony	
	Mal$^-$	Xyl$^-$	Mal$^-$	Xyl$^-$
rec$^+$	2/4,080	4/4,080	1/28,301	1/28,301
recG	25/6,427	19/6,427	1/24,036	3/24,036

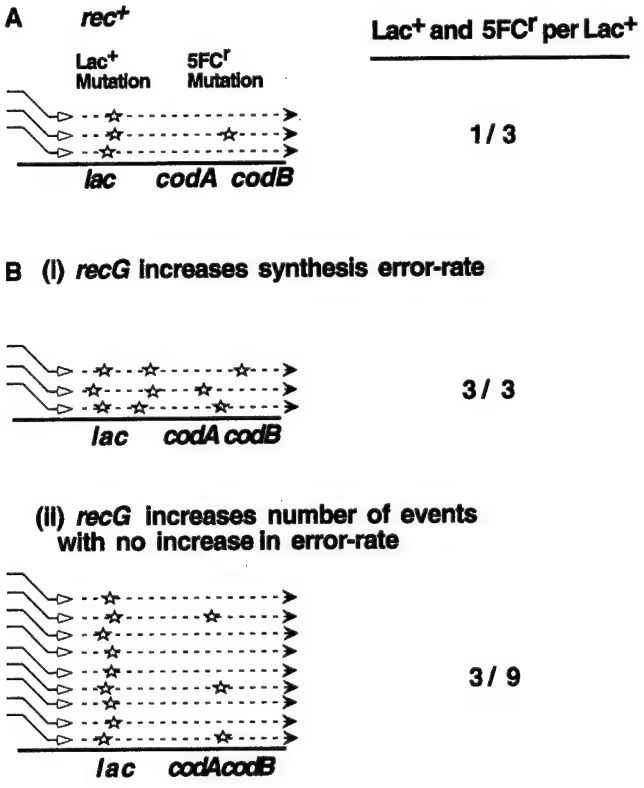


FIGURE 3.—Models for mutation at a locus linked to *lac* on the F′. (A) Proposal that mutation at *lac* and the linked *codAB* locus result from a common DNA replication event. *lac* and *codA codB* are located near each other on the F′. Among all selected mutational events that give rise to Lac$^+$ colonies, some frequency of the time (1/3, as a simple model) an unselected mutation in *codA* or *codB* also occurs, giving rise to a Lac$^+$ FC^r colony. (B) Two models to account for the increase in Lac$^+$ mutations in a *recG* strain. (i) If *recG* increased the error rate of DNA synthesis (for example by disabling DNA polymerase proofreading or postsynthesis mismatch repair), then the number of mutations per base synthesized would increase. As a consequence, the frequency of unselected mutations at *codA, codB* would increase among Lac$^+$ colonies. This is not observed (Table 3). (ii) If *recG* increases the number of synthesis events without affecting the error rate, then the ratio of Lac$^+$ 5FC^r (3/9) will not change compared with the *rec*$^+$ situation (1/3). This latter model is supported by the data in Table 3. (- - -) Newly synthesized DNA.

1997). These F′-linked mutations associated with Lac$^+$ could result from recombination-dependent mutation, but might not show increased mutability in *recG* or *recD*, if the recombination event that leads to their formation is the same event responsible for mutation at *lac* (Figure 3). For example, mutations at *lac* and the nearby locus *codAB* might occur via polymerase errors made during the same act of DNA synthesis, from the same recombinational DNA repair event (Figure 3A). If so, their coincident frequency would not be increased by conditions that increase only the number of recombination (and synthesis) events, without increasing the error rate per base synthesized (Figure 3Bii). Both *recD* and *recG* null alleles are expected to increase replicative strand-exchange intermediates (discussed above) and not error rate per base synthesized, as neither has a general mutator phenotype (HARRIS *et al.* 1994, 1996; FOSTER *et al.* 1996).

Unselected mutations at *codAB*, on the F′, were assayed in Lac$^+$ stationary-phase mutants (MATERIALS AND METHODS; TORKELSON *et al.* 1997). In the case of *recG* cells (Table 3), there is a 2-fold increase in the frequency of *codAB* mutation in Lac$^+$ mutants, whereas Lac$^+$ mutation itself was increased 26-fold. These physically linked sites do not show elevated coincident mutation, as the unlinked chromosomal and *lac* sites do (Tables 1 and 2; Figure 2). This implies that the recombination events that generate Lac$^+$ and *codAB*$^-$ mutations are not independent events.

We infer from these data that Lac$^+$ and chromosomal mutations occur during independent events and that both events are stimulated in *recD* and *recG* cells. One implication of these results is that *lac* and the sites mutated in the chromosome do not need to be joined physically (as they would be in an Hfr cell) during chromosomal hypermutation (discussed below).

In *recD*, mutation at *codAB* was increased just over twofold relative to *rec*$^+$, whereas Lac$^+$ mutation increased fourfold (Table 3). Recall that a twofold increase in secondary mutation frequency in *recD* was also seen for chromosomal loci (Table 1 and Figure 2). This suggests that the absence of RecD affects *codAB* and *lac* independently at least for some of the mutation events. Possible bases for these results are discussed below.

DISCUSSION

The results reported here can be summarized as follows:

1. Absence of either RecD or RecG increases concurrent mutation of chromosomal sites in Lac$^+$ stationary-phase mutants (Table 1 and Figure 2).
2. This increase is similar to the increase in Lac$^+$ mutation in *recD* and *recG* strains (Figure 2).
3. The increase in chromosomal mutation frequency is specific to cells that experienced a Lac$^+$ mutation and is not seen in Lac$^-$ starved cells (at least in the case of *recG*; Table 2).

   Because both *recD* and *recG* are predicted to promote strand-exchange recombination intermediates leading to replication (Figure 1, and reviewed above), these data support models in which (some) sites on the bacterial chromosome are accessible to recombination-promoted mutation in stationary phase.
4. The increase in mutability in *recG* is observed at chromosomal sites but not at *codAB* on the F′ (Table 3). This implies that mutations at sites linked to *lac* do not usually occur independently of the Lac$^+$ mutation event. This also implies that *lac* and chromosomal sites are not linked during mutation of chromosomal

TABLE 3

Frequencies of coincident mutation at F′-linked loci *codAB* and *lac* in *recD* and *recG* strains

rec genotype	$Lac^+/10^8$ cells plated (n)[a]	Fold increase (rec/rec^+)[b]	$5FC^r$ mutants/Lac^+ adaptive revertants screened	Fold increase (rec/rec^+)[c]
rec^+	15.6 (5,587)	1.0	18/5,170	1.0
recD	68.72 (11,522)	4.4	86/10,906	2.2
recG	406.3 (8,429)	26	58/8,394	2.0

[a] n, number of Lac^+ mutant colonies scored.
[b] Fold increase in the frequency of Lac^+ reversion.
[c] Fold increase in the frequency of unselected $5FC^r$ mutation in the F′.

sites. We suggest that the same recombination events that lead to Lac^+ mutation also lead to mutation of nearby genes, perhaps in the same DNA recombination-replication event. Evidence that recombination events promote DNA replication directly is reported elsewhere (Motamedi *et al.* 1999 and references reviewed therein; see also Courcelle *et al.* 1997; Kogoma 1997; Liu *et al.* 1999 for further discussion).

Recombination-promoted mutation in the bacterial chromosome: It was suggested that recombination-dependent stationary-phase mutation might be confined to sex plasmids because mutations at *lac* require F′ transfer (Tra) proteins (Foster and Trimarchi 1995a; Galitski and Roth 1995), though not actual transfer (Foster and Trimarchi 1995b; Radicella *et al.* 1995; Rosenberg *et al.* 1995), and because the *lac* operon on the chromosome is cold for recombination-dependent mutation (Foster and Trimarchi 1995a; Radicella *et al.* 1995; Rosche and Foster 1999; M.-J. Lombardo and S. M. Rosenberg, unpublished results). Previous evidence arguing against F′ specificity included, first, hypermutation of chromosomal genes during Lac^+ adaptive mutation (Torkelson *et al.* 1997; Rosche and Foster 1999; this study) and, second, the demonstration of chromosomal hot and cold spots for mutation (Rosenberg 1997; Torkelson *et al.* 1997), which can explain why not all chromosomal sites mutate recombinationally.

The demonstrations that *recG* and *recD* promote coincident chromosomal mutation (Figure 2; Tables 1 and 2) suggest that chromosomal sites are susceptible to recombination-dependent mutation. Note that we cannot test recombination dependence directly because blocking recombination via, *e.g.*, loss of RecA, RecB, or RuvA, B, or C functions abolishes stationary-phase Lac^+ mutation (Harris *et al.* 1994, 1996; Foster *et al.* 1996), and we have scored chromosomal mutations only in cells that are also Lac^+. Thus, although further, direct evidence is required to demonstrate conclusively that recombination-dependent mutation occurs in the *E. coli* chromosome, the current information is most easily explained by such a model.

Independence of Lac^+ and coincident chromosomal mutations: The finding that the coincident mutation frequency of *lac* and chromosomal sites increases in *recD* and *recG* cells (Tables 1 and 2; Figure 2) implies that the mutation frequency at each site is increased by these alleles. These results bear on the possibility that although it occurs in the chromosome, hypermutation during Lac reversion might actually require integration of the F′ into the chromosome. This occurs when Hfr chromosomes form (*e.g.*, Lloyd and Low 1996). We found previously that Lac^+ mutants carrying chromosomal mutations are not enriched for Hfr's (Lombardo *et al.* 1999b). However, we could not rule out the possibility that chromosomal mutations form in short-lived Hfr cells, which subsequently re-form the F′ (Lombardo *et al.* 1999b). The mostly independent stimulation of mutation in chromosomal and *lac* genes by *recD* and *recG* (Tables 1 and 2; Figure 2) does not support such models.

Site-specificity and the role of the F′: We have suggested that the key feature that allows some sites, and not others, to mutate recombinationally is occurrence of DNA DSBs at which RecBCD loads (Harris *et al.* 1994; Rosenberg *et al.* 1995; Rosenberg 1997; Torkelson *et al.* 1997). In this view, Tra proteins activate the F′ by nicking the origin of transfer (Rosenberg *et al.* 1995), and hot and cold sites on the chromosome correspond with sites that are more or less susceptible to DSBs (reviewed by Rosenberg 1997).

Although the results presented here suggest that the F is not needed *in cis* with the DNA that mutates (discussed above), it remains possible that *trans*-acting functions encoded by the F are required for mutation of chromosomal genes. The F encodes several proteins that interact with DNA, including its own single-strand DNA binding protein, a topoisomerase-like double-strand endonuclease, components that modify the bacterial SOS response, and many of the transfer proteins (reviewed by Bagdasarian *et al.* 1992; Frost *et al.* 1994; Yarmolinsky 1995; Firth *et al.* 1996). Whether recombination-dependent stationary-phase mutation and hypermutation of unselected genes can occur in the absence of sex plasmids is not yet known (see *Note added in proof*).

***recD* and coincident mutation in the F′ and chromosome:** *recD* null mutants are hyperrecombinagenic (Chaudhury and Smith 1984; Amundsen *et al.* 1986; Biek and Cohen 1986; Thaler *et al.* 1989), hypermutable in recombination-dependent stationary-phase Lac mutation (Harris *et al.* 1994; Rosenberg *et al.* 1994), and recently have been seen to increase F′ copy number relative to the chromosome (Foster and Rosche 1999). The stationary-phase hypermutation at *lac* in *recD* cells might have resulted from hyperrecombination in *recD* cells (Harris *et al.* 1994) or from more *lac* copies available for mutation in those cells (Foster and Rosche 1999) or from both. The finding that chromosomal gene mutability increases about as much as *lac* does in *recD* cells supports the recombinational idea and does not support the idea of an effect based purely on increased F′ copy number relative to the chromosome.

A perplexing result is that, unlike *recG*, the *recD* effect on chromosomal and F′ sites was similar (Tables 1 and 2). This could indicate a global (stationary-phase specific; Harris *et al.* 1994) twofold mutator activity in *recD* strains. However, another interpretation is possible. Loss of the RecD subunit changes RecBCD enzyme (Amundsen *et al.* 1986; Palas and Kushner 1990) and prevents Chi recognition by the enzyme (Chaudhury and Smith 1984; Thaler *et al.* 1989). Whereas most recombination models include RecBCD-mediated digestion of DNA from a double-strand end up to a Chi site followed by recombination at Chi (*e.g.*, Rosenberg and Hastings 1991; Myers and Stahl 1994; Anderson and Kowalczykowski 1997), in *recD* (exonuclease-defective) cells, the RecBC(D^-) enzyme promotes recombination immediately at the DNA end at which it loads (Thaler *et al.* 1989). This would change the position of strand-invasion events and, in models in which recombination primes replication, would alter the positions of synthesis tracts (Figure 4). Two loci might be synthesized on the same tract in *rec*$^+$ cells and on different tracts in *recD* cells (Figure 4), leading to uncoupling of *lac* and *codAB* mutation in *recD* cells.

Implications for the hypermutable subpopulation: *1. Recombination and the hypermutable subpopulation:* Previously, *lac* and an F′-borne gene were observed to show no increase in coincident mutation in *recG* cells, leading to the suggestion that *recG* somehow increases the size of the hypermutable subpopulation, rather than the mutability per subpopulation cell (Foster 1997). Our results for the F′ (Table 3) agree with those reported previously (Foster 1997). However, the data we have obtained on chromosomal site mutability (Table 1 and Figure 2) do not support the idea that *recG* increases subpopulation size, but rather imply that the mutability of subpopulation cells is increased by promoting strand-exchange intermediates. We suggest that at linked sites, the secondary mutation event and the primary Lac$^+$ mutation event are not independent, such that their coincident mutation frequency does not reflect the mutability per cell.

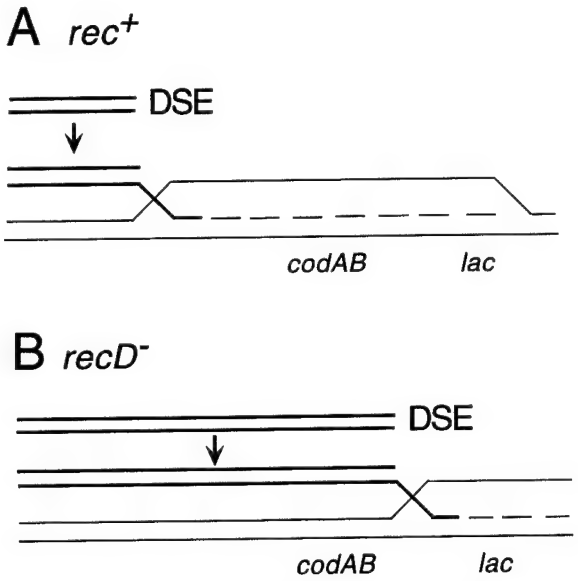


Figure 4.—DSEs and therefore DNA synthesis tracts primed in double-strand break repair would fall in different places in *rec*$^+$ and *recD*$^-$ cells. This is so because the RecBCD enzyme is a double-strand DNA exonuclease whereas the RecBC(D^-) enzyme lacks exonuclease activity, but is recombination-proficient (Chaudhury and Smith 1984; Amundsen *et al.* 1986; Biek and Cohen 1986). Dashed lines represent newly synthesized DNA. See text for discussion.

2. How many mutable subpopulations? Torkelson *et al.* (1997) reported that single (Lac$^+$), double (Lac$^+$ plus an additional mutation), and triple mutants (Lac$^+$ plus two additional mutations) fit a Poisson distribution if a mutation rate of 5×10^{-3} mutations per cell per day occurred in 10^{-4} to 10^{-5} of the cells of the whole population. The ability to fit the data to a Poisson distribution was taken to imply that mutation to Lac$^+$ and the formation of associated mutations occur at about the same frequency. This is compatible with the hypothesis that Lac$^+$ and associated mutations occur by the same mechanism and arise from the same subpopulation, but does not exclude the possibility that there are two or more mechanisms affecting different but overlapping subpopulations. A different data set and method of calculation led to the conclusion that only 10% of the Lac$^+$ mutations result from the hypermutating subpopulation that gives rise to the secondary mutations (Rosche and Foster 1999). However, the data on associated mutations were few, such that with 95% confidence limits applied to them, as many as 98% of the Lac$^+$ mutants could have arisen from the hypermutable subpopulation.

Nevertheless, the general concept of different but overlapping subpopulations may be applied to the results presented here. The *recD* and *recG* mutations might increase the size of the subpopulation undergoing mutation to Lac$^+$ such that the subpopulation now includes

a higher proportion of those cells of the subpopulation that gives rise to associated mutations. This would have the effect of increasing the frequency of associated mutations among the Lac^+ mutants without increasing the mutation rate in the hypermutating subpopulation. Invoking two populations and two mechanisms is a more complicated and thus less attractive model.

3. Subpopulation size and mutation rate: The proposed mutation rate of 5×10^{-3} mutations per cell per day of TORKELSON *et al.* (1997) may seem lethally high, and yet no net cell death is observed (CAIRNS and FOSTER 1991, and many subsequent references). It should be noted, however, that first, even massive death of a subpopulation of 10^{-5} of the cells would be unnoticeable when measuring cell viability and, second, because only some (hot) sites are mutable (see discussion of hot and cold sites above in ROSENBERG 1997), many essential genes may be spared, so death might not occur (supported by data of FOSTER 1997).

Significance: The findings reported here suggest that recombination-dependent stationary-phase mutation is a mechanism of genetic change under stress that can alter at least some of the cell's primary genetic reserve, the chromosomal genes. This inference will hold whether or not components on the F′ are found to be required for the chromosomal hypermutation. Sex plasmids are natural genetic elements and if they provide such conditional mutability to their hosts, this could be an advantageous, selected feature for their host cells.

Several aspects of recombination-dependent stationary-phase mutation may also be general to other organisms and circumstances. Mutation promoted by DSB-repair recombination in yeast has been demonstrated (STRATHERN *et al.* 1995; HOLBECK and STRATHERN 1997), as has recombinational involvement in mutation in vertebrates including mammals (reviewed by MAIZELS 1995; HARRIS *et al.* 1999b). Findings suggestive of this association abound in many organisms (DEMEREC 1962, 1963; MAGNI and VON BORSTEL 1962; PASZEWSKI and SURZYCKI 1964; ESPOSITO and BRUSCHI 1993). Additionally, the MMR system, which becomes limiting during stationary-phase mutation (HARRIS *et al.* 1997b, 1999a), is conserved in eubacteria and eukaryotes. Its loss of function is also a powerful force of genetic change in other organisms (reviewed by RADMAN *et al.* 1995; KOLODNER 1996; MODRICH and LAHUE 1996), and its transient diminution would be potentially more important in multicellular organisms that suffer more drastic consequences from mutagenesis of component cells. The mechanism of action, control, and scope of this stationary-phase mutation mechanism in *E. coli* will illuminate a path toward understanding conditional mutagenesis, programmed or accidental, in all of these systems.

We thank R. S. Harris, M.-J. Lombardo, and J. Petrosino for comments on the manuscript. This work was supported by National Institutes of Health grants R01 GM53158 and R01 AI43917, and in part by an Alberta Cancer Board Postdoctoral Fellowship (H.J.B.), by a D.O.D. Breast Cancer Research Program Scholarship (G.J.M.), and by Alberta Heritage Foundation for Medical Research Senior Scholarship and Medical Research Council of Canada Scientist awards to S.M.R.

Note addeed in proof: Recent work of GODOY *et al.* (2000) both confirms previous findings of chromosomal hypermutation during Lac^+ adaptive mutation (TORKELSON *et al.* 1997; ROSCHE and FOSTER 1999) and indicates that there is indeed an F′-supplied function that promotes stationary-phase mutation. As discussed above, the results presented here imply that any F′-related function would act *in trans* in mutation, not via Hfr formation (above, and LOMBARDO *et al.* 1999b).

LITERATURE CITED

AL-DEIB, A. A., A. A. MAHDI and R. G. LLOYD, 1996 Modulation of recombination and DNA repair by the RecG and PriA helicases of *Escherichia coli* K-12. J. Bacteriol. **178:** 6782–6789.

AMUNDSEN, S. K., A. F. TAYLOR, A. G. CHAUDHURY and G. R. SMITH, 1986 *recD*: the gene for an essential third subunit of exonuclease V. Proc. Natl. Acad. Sci. USA **83:** 5558–5562.

ANDERSON, D. G., and S. C. KOWALCZYKOWSKI, 1997 The recombination hot spot chi is a regulatory element that switches the polarity of DNA degradation by the RecBCD enzyme. Genes Dev. **11:** 571–581.

BAGDASARIAN, M., A. BAILONE, J. F. ANGULO, P. SCHOLZ, M. BAGDASARIAN *et al.*, 1992 PsiB, an anti-SOS protein, is transiently expressed by the F sex factor during its transmission to an *Escherichia coli* K-12 recipient. Mol. Microbiol. **6:** 885–893.

BENSON, S., 1997 Adaptive mutation: a general phenomenon or a special case? Bioessays **19:** 9–11.

BIEK, D. P., and S. N. COHEN, 1986 Identification and characterization of *recD*, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. J. Bacteriol. **167:** 594–603.

BRIDGES, B. A., 1997 Hypermutation under stress. Nature **387:** 557–558.

CAIRNS, J., and P. L. FOSTER, 1991 Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:** 695–701.

CAIRNS, J., J. OVERBAUGH and S. MILLER, 1988 The origin of mutants. Nature **335:** 142–145.

CAPORALE, L. H. (Editor), 1999 *Molecular Strategies in Biological Evolution*. Annals of the New York Academy of Sciences, New York.

CHAUDHURY, A. M., and G. R. SMITH, 1984 A new class of *Escherichia coli recBC* mutants: implications for the role of RecBC enzyme in homologous recombination. Proc. Natl. Acad. Sci. USA **81:** 7850–7854.

COURCELLE, J., C. CARSWELL-CRUMPTON and P. C. HANAWALT, 1997 RecF and RecR are required for resumption of replication at DNA replication forks in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **94:** 3714–3719.

CULOTTA, E., 1994 Evolutionary biology: a boost for "adaptive" mutation. Science **265:** 318–319.

DEMEREC, M., 1962 "Selfers" attributed to unequal crossovers in *Salmonella*. Proc. Natl. Acad. Sci. USA **48:** 1695–1704.

DEMEREC, M., 1963 Selfer mutants of *Salmonella typhimurium*. Genetics **48:** 1519–1531.

DRAKE, J. W., 1991 Spontaneous mutation. Annu. Rev. Genet. **25:** 125–146.

ESPOSITO, M. S., and C. V. BRUSCHI, 1993 Diploid yeast cells yield homozygous spontaneous mutations. Curr. Genet. **23:** 430–434.

FIRTH, N., K. IPPEN-IHLER and R. H. SKURRAY, 1996 Structure and function in the F factor and mechanism of conjugation, pp. 2377–2401 in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, edited by F. C. NEIDHARDT, R. CURTISS III, J. L. INGRAHAM, E. C. C. LIN, K. B. LOW, B. MAGASANIK, W. S. REZNIKOFF, M. RILEY, M. SCHAECHTER and H. E. UMBAGER. ASM Press, Washington, DC.

FOSTER, P. L., 1993 Adaptive mutation: the uses of adversity. Annu. Rev. Microbiol. **47:** 467–504.

FOSTER, P. L., 1997 Nonadaptive mutations occur in the F′ episome during adaptive mutation conditions in *Escherichia coli*. J. Bacteriol. **179:** 1550–1554.

FOSTER, P. L., and W. A. ROSCHE, 1999 Increased episomal replication accounts for the high rate of adaptive mutation in *recD* mutants of *Escherichia coli*. Genetics **152:** 15–30.

FOSTER, P. L., and J. M. TRIMARCHI, 1994 Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. Science **265:** 407–409.

FOSTER, P. L., and J. M. TRIMARCHI, 1995a Adaptive reversion of an episomal frameshift mutation in *Escherichia coli* requires conjugal functions but not actual conjugation. Proc. Natl. Acad. Sci. USA **92:** 5487–5490.

FOSTER, P. L., and J. M. TRIMARCHI, 1995b Conjugation is not required for adaptive reversion of an episomal frameshift mutation in *Escherichia coli*. J. Bacteriol. **177:** 6670–6671.

FOSTER, P. L., G. GUDMUNDSSON, J. M. TRIMARCHI, H. CAI and M. F. GOODMAN, 1995 Proofreading-defective DNA polymerase II increases adaptive mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **92:** 7951–7955.

FOSTER, P. L., J. M. TRIMARCHI and R. A. MAURER, 1996 Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. Genetics **142:** 25–37.

FROST, L. S., K. IPPEN-IHLER and R. A. SKURRAY, 1994 Analysis of the sequence and gene products of the transfer region of the F sex factor. Microbiol. Rev. **58:** 162–210.

GALITSKI, T., and J. R. ROTH, 1995 Evidence that F′ transfer replication underlies apparent adaptive mutation. Science **268:** 421–423.

GALITSKI, T., and J. R. ROTH, 1996 A search for a general phenomenon of adaptive mutability. Genetics **143:** 645–659.

GODOY, V. G., F. S. GIZATULLIN and M. S. FOX, 2000 Some features of mutability of bacteria during nonlethal selection. Genetics **154:** 49–59.

HALL, B. G., 1990 Spontaneous point mutations that occur more often when advantageous than when neutral. Genetics **126:** 5–16.

HALL, B. G., 1993 Selection-induced mutations. Curr. Opin. Genet. Dev. **2:** 943–946.

HALL, B. G., 1995 Genetics of selection-induced mutations: I. *uvrA*, *uvrB*, *uvrC*, and *uvrD* are selection-induced specific mutator loci. J. Mol. Evol. **40:** 86–93.

HARRIS, R. S., S. LONGERICH and S. M. ROSENBERG, 1994 Recombination in adaptive mutation. Science **264:** 258–260.

HARRIS, R. S., K. J. ROSS and S. M. ROSENBERG, 1996 Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. Genetics **142:** 681–691.

HARRIS, R. S., H. J. BULL and S. M. ROSENBERG, 1997a A direct role for DNA polymerase III in adaptive reversion of a frameshift mutation in *Escherichia coli*. Mutat. Res. **375:** 19–24.

HARRIS, R. S., G. FENG, K. J. ROSS, R. SIDHU, C. THULIN *et al.*, 1997b Mismatch repair protein MutL becomes limiting during stationary-phase mutation. Genes Dev. **11:** 2426–2437.

HARRIS, R. S., G. FENG, K. J. ROSS, R. SIDHU, C. THULIN *et al.*, 1999a Mismatch repair is diminished during stationary-phase mutation. Mutat. Res. **437:** 51–60.

HARRIS, R. S., Q. KONG and N. MAIZELS, 1999b Somatic hypermutation and the three R's: repair, replication and recombination. Mutat. Res. **436:** 157–178.

HOLBECK, S. L., and J. N. STRATHERN, 1997 A role for REV3 in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae*. Genetics **147:** 1017–1024.

KOGOMA, T., 1997 Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. Microbiol. Mol. Biol. Rev. **61:** 212–238.

KOLODNER, R., 1996 Biochemistry and genetics of eukaryotic mismatch repair. Genes Dev. **10:** 1433–1442.

KUZMINOV, A., 1995 Collapse and repair of replication forks in *Escherichia coli*. Mol. Microbiol. **16:** 373–384.

LIU, J., L. XU, S. J. SANDLER and K. J. MARIANS, 1999 Replication fork assembly at recombination intermediates is required for bacterial growth. Proc. Natl. Acad. Sci. USA **96:** 3552–3555.

LLOYD, R. G., and K. B. LOW, 1996 Homologous recombination, pp. 2236–2255 in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, edited by F. C. NEIDHARDT, R. CURTISS III, J. L. INGRAHAM, E. C. C. LIN, K. B. LOW, B. MAGASANIK, W. S. REZNIKOFF, M. RILEY, M. SCHAECHTER and H. E. UMBAGER. ASM Press, Washington, DC.

LOMBARDO, M.-J., and S. M. ROSENBERG, 1999 Hypermutation in stationary-phase *E. coli*: tales from the *lac* operon. J. Genet. **78:** 13–20.

LOMBARDO, M.-J., R. S. HARRIS and S. M. ROSENBERG, 1999a Stressful lifestyle-associated mutation in microorganisms, pp. 71–90 in *Plant Responses to Environmental Stresses, From Phytohormones to Genome Reorganization*, edited by H. R. LERNER. Marcel Dekker, New York.

LOMBARDO, M.-J., J. TORKELSON, H. J. BULL, G. J. MCKENZIE and S. M. ROSENBERG, 1999b Mechanisms of genome-wide hypermutation in stationary phase. Ann. NY Acad. Sci. **870:** 275–289.

LONGERICH, S., A. M. GALLOWAY, R. S. HARRIS, C. WONG and S. M. ROSENBERG, 1995 Adaptive mutation sequences reproduced by mismatch repair deficiency. Proc. Natl. Acad. Sci. USA **92:** 12017–12020.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MAENHAUT-MICHEL, G., and J. A. SHAPIRO, 1994 The roles of starvation and selective substrates in the emergence of *araB-lacZ* fusion clones. EMBO J. **13:** 5229–5239.

MAENHAUT-MICHEL, G., C. E. BLAKE, D. R. LEACH and J. A. SHAPIRO, 1997 Different structures of selected and unselected *araB-lacZ* fusions. Mol. Microbiol. **23:** 133–145.

MAGNI, G. E., and R. C. VON BORSTEL, 1962 Different rates of spontaneous mutation during mitosis and meiosis in yeast. Genetics **47:** 1097–1108.

MAIZELS, N., 1995 Somatic hypermutation: how many mechanisms diversify V region sequences? Cell **83:** 9–12.

MCGLYNN, P., A. A. AL-DEIB, J. LIU, K. J. MARIANS and R. G. LLOYD, 1997 The DNA replication protein PriA and the recombination protein RecG. J. Mol. Biol. **270:** 212–221.

MCKENZIE, G. J., M.-J. LOMBARDO and S. M. ROSENBERG, 1998 Recombination-dependent mutation in *Escherichia coli* occurs in stationary phase. Genetics **149:** 1563–1565.

MICHEL, B., S. D. EHRLICH and M. UZEST, 1997 DNA double-strand breaks caused by replication arrest. EMBO J. **16:** 430–438.

MODRICH, P., and R. LAHUE, 1996 Mismatch repair in replication fidelity, genetic recombination, and cancer. Annu. Rev. Biochem. **65:** 101–133.

MOTAMEDI, M., S. K. SZIGETY and S. M. ROSENBERG, 1999 Double-strand break-repair in *E. coli*: physical evidence for a replication mechanism *in vivo*. Genes Dev. **13:** 2889–2903.

MYERS, R. S., and F. W. STAHL, 1994 χ and RecBCD enzyme of *Escherichia coli*. Annu. Rev. Genet. **28:** 49–70.

NINIO, J., 1991 Transient mutators: a semiquantitative analysis of the influence of translation and transcription errors on mutation rates. Genetics **129:** 957–962.

PALAS, K. M., and S. R. KUSHNER, 1990 Biochemical and physical characterization of exonuclease V from *Escherichia coli*: comparison of the catalytic activities of the RecBC and RecBCD enzymes. J. Biol. Chem. **265:** 3447–3454.

PASZEWSKI, A., and S. SURZYCKI, 1964 "Selfers" and high mutation rate during meiosis in *Ascobolus immersus*. Nature **204:** 809.

PENNISI, E., 1998 How the genome readies itself for evolution. Science **281:** 1131–1134.

PETERS, J. E., I. M. BARTOSZYK, S. DHEER and S. A. BENSON, 1996 Redundant homosexual transfer facilitates selection-induced reversion of plasmid mutations. J. Bacteriol. **178:** 3037–3043.

RADICELLA, J. P., P. U. PARK and M. S. FOX, 1995 Adaptive mutation in *Escherichia coli*: a role for conjugation. Science **268:** 418–420.

RADMAN, M., I. MATIC, J. A. HALLIDAY and F. TADDEI, 1995 Editing DNA replication and recombination by mismatch repair: from bacterial genetics to mechanisms of predisposition to cancer in humans. Philos. Trans. R. Soc. Lond. B Biol. Sci. **347:** 97–103.

ROSCHE, W. A., and P. L. FOSTER, 1999 The role of transient hypermutators in adaptive mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **96:** 6862–6867.

ROSENBERG, S. M., 1994 In pursuit of a molecular mechanism for adaptive mutation. Genome **37:** 893–899.

ROSENBERG, S. M., 1997 Mutation for survival. Curr. Opin. Genet. Dev. **7:** 829–834.

ROSENBERG, S. M., and P. J. HASTINGS, 1991 The split-end model for homologous recombination at double-strand breaks and at Chi. Biochimie **73:** 385–397.

ROSENBERG, S. M., and M. R. MOTAMEDI, 1999 Homologous recombination during bacterial conjugation, in *Embryonic Encyclopedia of Life Sciences*. Nature Publishing Group, London (http://www.els.net).

ROSENBERG, S. M., S. LONGERICH, P. GEE and R. S. HARRIS, 1994 Adaptive mutation by deletions in small mononucleotide repeats. Science **265:** 405–407.

ROSENBERG, S. M., R. S. HARRIS and J. TORKELSON, 1995 Molecular handles on adaptive mutation. Mol. Microbiol. **18:** 185–189.

ROSENBERG, S. M., R. S. HARRIS, S. LONGERICH and A. M. GALLOWAY, 1996 Recombination-dependent mutation in non-dividing cells. Mutat. Res. **350:** 69–76.

ROSENBERG, S. M., C. THULIN and R. S. HARRIS, 1998 Transient and heritable mutators in adaptive evolution in the lab and in nature. Genetics **148:** 1559–1566.

SEIGNEUR, M., V. BIDNENKO, S. D. EHRLICH and B. MICHEL, 1998 RuvAB acts at arrested replication forks. Cell **95:** 419–430.

SHAPIRO, J. A., 1997 Genome organization, natural genetic engineering and adaptive mutation. Trends Genet. **13:** 98–104.

STRATHERN, J. N., B. K. SHAFER and C. B. MCGILL, 1995 DNA synthesis errors associated with double-strand-break repair. Genetics **140:** 965–972.

SYMONDS, N. D., 1993 Francis Ryan and the origins of directed mutagenesis. Mutat. Res. **285:** 9–12.

TADDEI, F., J. A. HALLIDAY, I. MATIC and M. RADMAN, 1997 Genetic analysis of mutagenesis in aging *Escherichia coli* colonies. Mol. Gen. Genet. **256:** 277–281.

THALER, D. S., 1994 The evolution of genetic intelligence. Science **264:** 224–225.

THALER, D. S., E. SAMPSON, I. SIDDIQI, S. M. ROSENBERG, L. C. THOMASON *et al.*, 1989 Recombination of bacteriophage λ in *recD* mutants of *Escherichia coli*. Genome **31:** 53–67.

TORKELSON, J., R. S. HARRIS, M.-J. LOMBARDO, J. NAGENDRAN, C. THULIN *et al.*, 1997 Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. EMBO J. **16:** 3303–3311.

WHITBY, M. C., L. RYDER and R. G. LLOYD, 1993 Reverse branch migration of Holliday junctions by RecG protein: a new mechanism for resolution of intermediates in recombination and DNA repair. Cell **75:** 341–350.

WHITBY, M. C., S. D. VINCENT and R. G. LLOYD, 1994 Branch migration of Holliday junctions: identification of RecG protein as a junction specific DNA helicase. EMBO J. **13:** 5220–5228.

WRIGHT, B. E., A. LONGACRE and J. M. REIMERS, 1999 Hypermutation in derepressed operons of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **96:** 5089–5094.

YARMOLINSKY, M. B., 1995 Programmed cell death in bacterial populations. Science **267:** 836–837.

Communicating editor: P. G. YOUNG

Molecular Cell, Vol. 7, 571–579, March, 2001, Copyright ©2001 by Cell Press

SOS Mutator DNA Polymerase IV Functions in Adaptive Mutation and Not Adaptive Amplification

Gregory J. McKenzie,* Peter L. Lee,*
Mary-Jane Lombardo,* P. J. Hastings,*
and Susan M. Rosenberg*†‡§
*Department of Molecular and Human Genetics
†Department of Biochemistry
‡Department of Molecular Virology and Microbiology
Baylor College of Medicine
Houston, Texas 77030-3411

Summary

Adaptive point mutation and amplification are induced responses to environmental stress, promoting genetic changes that can enhance survival. A specialized adaptive mutation mechanism has been documented in one *Escherichia coli* assay, but its enzymatic basis remained unclear. We report that the SOS-inducible, error-prone DNA polymerase (pol) IV, encoded by *dinB*, is required for adaptive point mutation in the *E. coli lac* operon. A nonpolar *dinB* mutation reduces adaptive mutation frequencies by 85% but does not affect adaptive amplification, growth-dependent mutation, or survival after oxidative or UV damage. We show that pol IV, together with the major replicase, pol III, can account for all adaptive point mutations at *lac*. The results identify a role for pol IV in inducible genetic change.

Introduction

Radman (1975), Echols (1981), and others have suggested that states of accelerated evolution might be induced in response to stress and that enzymes might be specialized for this purpose. The discoveries of adaptive point mutation in bacteria and yeast, and of adaptive amplification in bacteria (Hastings et al., 2000), support the idea of differentiated states of hastened genetic change (reviewed by Rosenberg, 2001). Adaptive mutation is a process of increased mutability that occurs in stationary phase starving cells and can confer mutations allowing survival. There are many assay systems for its study (reviewed in Rosenberg, 1997, 2001; Foster, 1999), but in only one so far has adaptive mutation been demonstrated to occur by a molecular mechanism different from spontaneous mutation in growing cells (and so to be a separate process). That assay measures reversion of a *lac* +1 frameshift allele carried on an F′ episome in *Escherichia coli* (Cairns and Foster, 1991). In the *lac* system, one distinct mechanism produces adaptive point mutations, conferring a Lac$^+$ phenotype via compensatory frameshift mutations. Also in the *lac* system, a separate adaptive response produces adaptive amplifications (Hastings et al., 2000, and references therein for previous studies of amplification in bacteria). In adaptive amplification, the leaky *lac* mutant gene is amplified to many copies such that sufficient β-galactosidase activity is produced for growth on lactose medium *without* acquisition of a Lac$^+$ point mutation. Adaptive point mutation and amplification are separate adaptive responses and are both different from Lac$^+$ mutation in growing cells.

The adaptive point mutation mechanism at *lac* can be summarized as follows. The adaptive mutations occur after exposure to lactose medium (McKenzie et al., 1998) and require homologous recombination proteins of the RecBCD double strand break repair (DSBR) system (Harris et al., 1994, 1996; Foster et al., 1996). DSBR is proposed to promote mutation by priming replication during which DNA polymerase errors occur (Harris et al., 1994). Whereas growth-dependent Lac$^+$ mutations are heterogeneous, the adaptive mutations are nearly all −1 deletions in small mononucleotide repeats (Foster and Trimarchi, 1994; Rosenberg et al., 1994), resembling DNA polymerase errors formed by a template slippage mechanism (Streisinger et al., 1966; reviewed in Ripley, 1990). The adaptive mutations accumulate during a transient period of mismatch repair protein deficiency during starvation (Longerich et al., 1995; Harris et al., 1997b, 1999). The adaptive mutants, once formed, bear high frequencies of unrelated mutations throughout their genomes, indicating that some or all of the adaptive mutants arise during a transient genome-wide hypermutability (Torkelson et al., 1997; Rosche and Foster, 1999; Bull et al., 2000a; Godoy et al., 2000; and see Bull et al., 2000b; Cairns, 2000 for further discussion). Finally, efficient recombination-dependent adaptive mutation requires a functional SOS response for upregulation of a protein(s) other than or in addition to RecA (McKenzie et al., 2000). One infers that both recombination and SOS are required because recombination genes are required that are not also required for an SOS response (Foster et al., 1996; Harris et al., 1996).

The enzymatic basis of the mutability underlying adaptive mutation at *lac* has not been elucidated fully. Either of two different (general) mechanisms seems possible. On the one hand, the postreplicative mismatch repair (MMR) system (reviewed by Modrich and Lahue, 1996) becomes limiting transiently during adaptive mutation (Harris et al., 1997b, 1999), and genetic evidence implicates the major replicative DNA polymerase, pol III, in adaptive mutation (Foster et al., 1995; Harris et al., 1997a). Therefore, a normal rate of DNA polymerase error could lead to mutability because of failure to correct those errors. On the other hand, the involvement of the SOS response suggests (among other possibilities) that special mutator enzymes controlled by SOS could be responsible (McKenzie et al., 2000). The *umuDC*-encoded mutator DNA polymerase (pol) V is not required (Cairns and Foster, 1991; McKenzie et al., 2000). This study examines the other SOS mutator polymerase, pol IV, encoded by *dinB*.

Pol IV is a poorly processive error-prone DNA polymerase (Wagner et al., 1999; but see Tang et al., 2000; Wagner et al., 2000) and a member of the large, newly elaborated DinB/UmuDC superfamily of DNA polymerases in bacteria, archaea, and eukaryotes (reviewed by

§To whom correspondence should be addressed (e-mail: smr@bcm.tmc.edu).

Friedberg et al., 2000). The discoveries of multiple DNA polymerases in all living organisms have raised the question of why cells have so many (e.g., five are known currently in *E. coli*). What are their functions? Some of the DinB/UmuDC polymerases are translesion polymerases known to promote DNA damage survival by allowing replication to bypass otherwise replication-blocking lesions. The human XP-V (xeroderma pigmentosum variant) tumor suppressor protein (of the Rad30 subfamily) and *E. coli* pol V (of the UmuDC subfamily) are examples. However, the function(s) of pol IV (DinB subfamily) and three of its mammalian homologs (Friedberg et al., 2000) have been elusive. Pol IV may participate in mutation of undamaged phage λ DNA during infection of irradiated *E. coli* (λ untargeted mutagenesis; Brotcorne-Lannoye and Maenhaut-Michel, 1986). Pol IV overproduction causes hypermutation including –1 frameshifts and some substitutions (Kim et al., 1997; Wagner and Nohmi, 2000). The purified pol IV enzyme makes similar errors (Wagner et al., 2000).

We shall report that pol IV is required for most adaptive point mutation at *lac*, but not for mutations in growing cells, survival of UV or oxidative damage, or adaptive amplification. Thus, one function of pol IV in *E. coli* involves environmentally inducible genetic change.

Results

Experimental Strategy

To test whether adaptive mutation occurs in cells lacking a functional DNA pol IV, encoded by *dinB*, we constructed isogenic *dinB*$^+$ and mutant strains. *dinB* is the first gene in an apparent operon of four damage-inducible (Courcelle et al., 2001) SOS genes: *dinB*, *yafN*, *yafO*, and *yafP*. The *yaf* genes have unknown functions, though YafN has homology to the anti-toxin of the *relBE* operon (Grønlund and Gerdes, 1999). All of these genes are likely to be inactivated by previously published null alleles of *dinB*: a deletion of *dinB* and part of *yafN* (Kim et al., 1997), and an insertion (Kenyon and Walker, 1980). To remove only pol IV function, we created a nonpolar null allele of *dinB* identical to *dinB10* (Wagner et al., 1999), which replaces a highly conserved amino acid (R49F), producing a mutant polymerase that is inactive in vitro and does not enhance mutation when overproduced in vivo. The *lac* frameshift–bearing strain carries two copies of the *dinB*$^+$ gene, one on the F′ and one in the chromosome (Experimental Procedures). We constructed strains carrying *dinB10* at both sites.

In adaptive mutation assays, Lac$^-$ cells are plated onto lactose medium and incubated for several days (Experimental Procedures). Lac$^+$ mutant colonies that appear early (about day 2) represent growth-dependent mutants formed before plating on lactose medium (Cairns and Foster, 1991; see Harris et al., 1999). Colonies that appear late (e.g., day 3–7) consist of a majority of adaptive point mutants and a minority of adaptive amplified clones, both formed after plating on lactose medium (McKenzie et al., 1998; Hastings et al., 2000).

Pol IV Is Required Specifically for Adaptive Point Mutation at *lac*

Replacement of both copies of *dinB*$^+$ with *dinB10* reduces adaptive mutation about 4-fold (Figure 1A), indicating that DNA pol IV function is required for most adaptive mutation in the *lac* system. This phenotype can be complemented with a single, ectopic chromosomal copy of *dinB*$^+$ (Figure 1B), indicating that the decrease in adaptive mutation is caused solely by the loss of pol IV, and not other genes in the putative *dinB* operon. We note that a single chromosomal copy of *dinB*$^+$ is sufficient for adaptive mutation at *lac* (Figure 1B), contrary to the suggestion that expression of the extra copy of *dinB* on the F′ might be required (Godoy et al., 2000). These results indicate a biological role for pol IV: it promotes adaptive mutation.

The amount of adaptive point mutation requiring pol IV is greater than is apparent from the total colony counts in Figure 1. About 42% of the day 5 (i.e., adaptive) Lac$^+$ colonies that remain in the pol IV-deficient strain carry amplified arrays of the leaky *lac*$^-$ allele rather than a point mutation, as compared with 9.5% for *dinB*$^+$ (Figure 1A). These classes were distinguished by their colony color after purification by streaking for single colonies onto rich X-gal medium (Experimental Procedures). The fact that amplified clones are about 40% of day 5 colonies in pol IV-deficient cells indicates that the reduction in adaptive point mutation in pol IV-deficient cells is actually about 85% (25% Lac$^+$ mutants seen, 60% of which are point mutants, leaves 15% point mutation remaining) (Figures 1A and 2B). Thus, the vast majority of the adaptive point mutation is pol IV dependent.

In addition, the data show that pol IV is not required for adaptive amplification. Amplified clones constitute ~10% of Lac$^+$ colonies in pol IV$^+$ cells (above) and ~40% of Lac$^+$ colonies in pol IV$^-$, in which the total number of Lac$^+$ colonies is reduced 4-fold (25% of that seen in pol IV$^+$). Thus, the number of amplified clones in pol IV$^-$ cells is approximately the same as in pol IV$^+$ (40% amplified of 25% total colonies equals 10%). Pol IV is therefore required specifically for adaptive point mutation and not for adaptive amplification.

To test whether pol IV is also required for growth-dependent mutation, we measured the mutation rate in *dinB*$^+$ and *dinB*$^-$ growing cells using fluctuation tests (in which mutant frequencies determined in multiple independent cultures are used to calculate rates; Experimental Procedures). To exclude adaptive mutants from the counts of growth-dependent Lac$^+$ mutants, we acquired ten independent Lac$^+$ mutant derivatives of the *dinB*$^+$ and *dinB10* strain. These were seeded at a known number of cells per plate onto lactose plates under exact experimental conditions, in parallel with the cultures in which growth-dependent mutants were being enumerated. These controls indicate the earliest possible time to count Lac$^+$ colonies for each cell genotype (the time at which the seeded Lac$^+$ control colonies become visible) (Harris et al., 1999). Failure to use these controls can give uninterpretable results, because both growth-dependent and adaptive mutants contribute to the colony counts from which mutation rates are calculated (Harris et al., 1994, 1996, 1997b, 1999). The results in Table 1 show that pol IV is not required for growth-dependent mutation at *lac*.

We find that pol IV mutation does not affect the rate of other growth-dependent mutations, including substitutions, frameshifts, and other mutations in growing

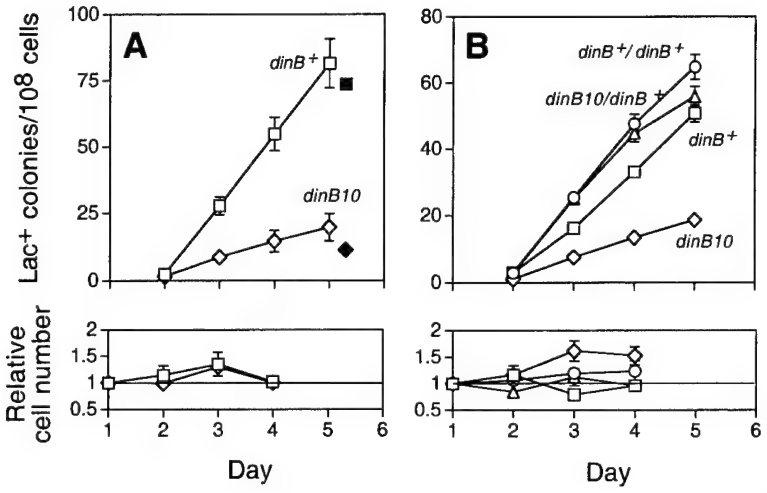


Figure 1. DNA Polymerase IV Is Required for Most Lac$^+$ Adaptive Mutation

(A) Total Lac$^+$ colonies are shown as open symbols. Lac$^+$ point mutants (see text) are plotted as filled symbols offset slightly from the day 5 point for clarity. The fraction of day 5 colonies carrying amplification (Experimental Procedures) was 9.5% (mean ± 2.6% SEM) in the *dinB*$^+$ and 42% (± 5.6%) in the *dinB10* strain.

(B) Decrease in mutation is complemented by a single, ectopic, chromosomal copy of *dinB*$^+$ controlled by its natural promoter. *dinB*$^+$ (open squares), *dinB10* (open diamonds), *dinB*$^+$ Δ*attB*::*dinB*$^+$ (open circles), and (Δ) *dinB10* Δ*attB*::*dinB*$^+$ (open triangles) strains SMR4562, SMR5830, SMR5834, and SMR5851, respectively. Means ± SEM (error bars) of ten independent cultures tested are shown (except for the filled symbols, mean ± SEM of four cultures). Where not visible, error bars are smaller than the plot symbol. Daily measurements of viable *lac*$^-$ cells on the plates (Relative cell number), shown normalized to the first day's count, show no net growth or death during the experiments (mean ± SEM, four cultures).

cells (Figure 3). We conclude that pol IV is required specifically for adaptive mutation.

Our results disagree with a previous study, in which a *dinB* mutation appeared to decrease the rate of growth-dependent Lac$^+$ mutation slightly (Strauss et al., 2000). The reason for the difference may be that the earlier study did not account for adaptive mutations. Alternatively, the small rate change may have been due to the use of a polar *dinB* allele, which also disrupted genes downstream of *dinB*.

Pol IV is also not required for survival of UV irradiation and oxidative damage caused by hydrogen peroxide. As seen in Figure 4, the *dinB10* mutant is indistinguishable from an isogenic *dinB*$^+$ strain in UV survival and hydrogen peroxide resistance. Control isogenic strains carrying the *lexA3*(Ind$^-$) mutation, blocking SOS gene induction, or a mutation in *xthA*, encoding an exonuclease required for repair of peroxide-induced damage (Demple et al., 1983), show reduced resistance, as expected.

SOS/LexA Induction Promotes Adaptive Point Mutation Wholly via Pol IV

Because pol IV is one of the genes induced by the SOS response (reviewed by Walker, 1996), we asked whether pol IV alone can account for the requirement for SOS induction in adaptive point mutation (Cairns and Foster, 1991; McKenzie et al., 2000; Figure 2A). If induction of additional SOS-induced genes were required, then *dinB10 lexA3*(Ind$^-$) cells (SOS noninducible due to an uncleavable mutant LexA repressor) should produce fewer adaptive mutations than *dinB10* cells. However, our experiments showed that the rate of adaptive mutation in both genetic backgrounds is the same (Figure 2B), implying that induction of SOS genes that act independently of pol IV is not required. Thus, genes such

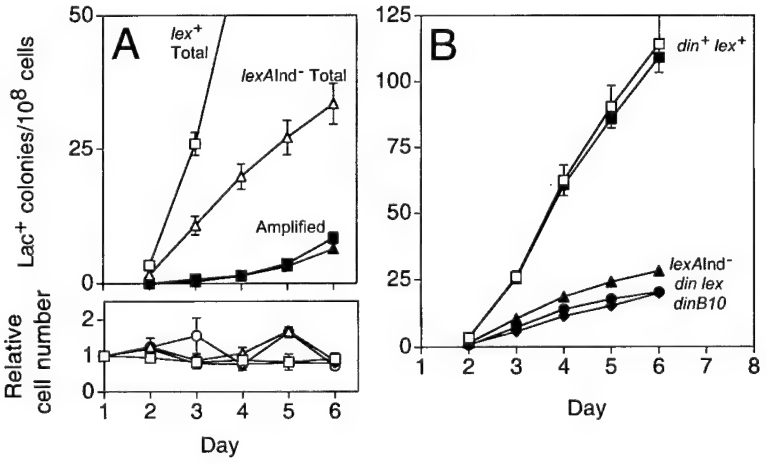


Figure 2. Different Roles of SOS Induction in Adaptive Amplification and Point Mutation

(A) Induction of the SOS/LexA regulon is not required for adaptive amplification. Total adaptive Lac$^+$ colonies (open symbols) are decreased by the *lexA3*(Ind$^-$) allele (open triangles), whereas the fraction amplified (filled symbols) is not. *lexA*$^+$ (squares) and *lexA3*(Ind$^-$) (triangles) strains SMR583 and SMR820, respectively.

(B) The contribution of SOS/LexA induction to adaptive point mutation is wholly via pol IV. Closed symbols display adaptive Lac$^+$ point mutants for *dinB*$^+$ *lexA*$^+$ (squares), *lexA3*(Ind$^-$) (triangles), *dinB10* (diamonds), and *dinB10 lexA3*(Ind$^-$) (circles) strains SMR583, SMR820, SMR5849, and SMR5850, respectively. This is the same experiment shown in (A) but with data from more of the strains tested in parallel shown, and point mutation displayed. Both sets of experiments were performed three times with similar results. In (B), the total adaptive Lac$^+$ colonies are also shown for the *dinB*$^+$ *lexA*$^+$ control strain (open squares). Means ± SEM (error bars) of ten independent cultures tested are shown (except for the filled symbols, mean ± SEM of four cultures). Where not visible, error bars are smaller than the plot symbol. Daily measurements of viable *lac*$^-$ cells on the plates (Relative cell number), shown normalized to the first day's count, show no net growth or death during the experiments (mean ± SEM, four cultures).

Table 1. DNA Polymerase IV Does Not Affect *lac* Frameshift Reversion in Growing Cells

Relevant Genotype	Experiment	Median Number of Mutants	Growth-Dependent Mutation Rate to Lac$^+$ (Mutations/Cell/Generation)	Mean (± SEM)
dinB$^+$	1	3.5	3.1×10^{-9}	$1.6 (\pm 0.3) \times 10^{-9}$
	2	12.8	1.9×10^{-9}	
	3	5.1	1.5×10^{-9}	
	4	5.0	1.8×10^{-9}	
dinB10	1	2.0	4.5×10^{-9}	$1.2 (\pm 0.3) \times 10^{-9}$
	2	7.4	1.2×10^{-9}	
	3	2.9	1.3×10^{-9}	
	4	3.0	1.1×10^{-9}	

Strains are *dinB*$^+$, SMR4562 and *dinB10*, SMR5830. See Experimental Procedures.

as the *recA*, *ruvA*, and *ruvB* recombination genes, which are required for adaptive mutation, appear to suffice at their noninduced (constitutive) levels. These results suggest that the requirement for SOS induction in adaptive point mutation (Cairns and Foster, 1991; McKenzie et al., 2000; Figure 2A) may be accounted for solely by pol IV.

Induction of LexA/SOS Genes Is Not Required for Adaptive Amplification

The SOS response was previously shown to be required for adaptive point mutation. We tested whether SOS-induced genes are also required for adaptive amplification. We found that blocking induction of the SOS/LexA regulon with the *lexA3*(Ind$^-$) allele (encoding an uncleavable LexA repressor protein; Mount et al., 1972; Lin and Little, 1989) decreases only point mutation, not adaptive amplification (Figure 2A, filled symbols). Thus, only adaptive point mutation, and not adaptive amplification, requires induction of LexA controlled genes, supporting the conclusion that these are separate pathways.

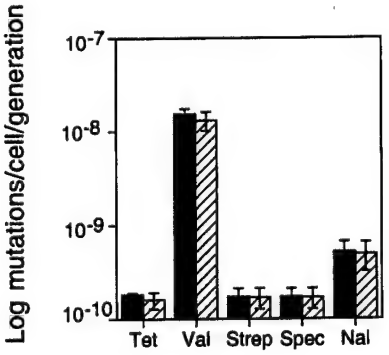


Figure 3. Rates of Frameshift and Substitution Mutations in *dinB*$^+$ and *dinB10* Cells during Growth

The various frameshift and substitution mutation assays (see LaRossa, 1996) follow: Val, a variety of different mutations in the isoleucine/valine biosynthesis genes, conferring valine resistance; Strep and Spec, substitution mutations in two ribosomal protein genes conferring streptomycin and spectinomycin resistance, respectively; Nal, substitution mutations in the *gyr* genes conferring nalidixic acid resistance; and Tet, reversion of a +1 frameshift mutation (4G to 5G, Experimental Procedures) in a chromosomal *tetA* gene conferring tetracycline resistance. This is similar to the 3G to 4G *lacI33* frameshift allele used in these adaptive mutation studies. *dinB*$^+$ (filled bars) and *dinB10* (hatched bars) strains are SMR4596 andSMR6049, respectively. Error bars, one SEM of three independent experiments.

Pol IV Contributes to –1 Deletions in a Variety of Mononucleotide Repeats

Lac$^+$ adaptive mutations are nearly all –1 deletions in small mononucleotide repeats (Foster and Trimarchi, 1994; Rosenberg et al., 1994). In the presence of wild-type *dinB*$^+$, most occur at a reversion hot spot (4Cs that include the +1 frameshift mutation inactivating *lac*), but a significant portion (about one-third) occurs at other mononucleotide repeats. We find that in the absence of pol IV, –1 frameshifts occur mostly at the hot spot (24/31 mutations sequenced, Figure 5), with other point mutations being larger insertions and deletions or not at mononucleotide repeats (Figure 5). The data imply that pol IV facilitates –1 deletions at many different mononucleotide repeats, mutations similar to the frameshift component of the error spectrum of the purified polymerase (Wagner et al., 1999).

Overlapping Roles of Pol III and Pol IV

Previous data suggested that pol III may play a role in adaptive point mutation. An antimutator pol III strain decreased the total number of adaptive Lac$^+$ mutations by about 4-fold (Foster et al., 1995; Harris et al., 1997a). In agreement with these results, we find that the antimutator pol III (encoded by *dnaE915*) reduces the number of adaptive point mutations by about 80% (Figure 6). Thus, neither pol IV mutation nor an antimutator pol III inhibits all adaptive point mutation. However, in cells carrying *dnaE915* and a defective pol IV (circles), adaptive point mutation is essentially abolished (Figure 6). These results show that the antimutator pol III decreases both the pol IV-dependent and the pol IV-independent adaptive point mutations, indicating overlapping roles for pol III and pol IV in this process (discussed below).

Discussion

The data presented in this paper imply that the SOS mutator DNA polymerase pol IV is a mutation-promoting enzyme required specifically for most (about 85% of) adaptive point mutation (Figure 1), but not for growth-dependent Lac$^+$ (Table 1) or other (Figure 3) mutation. Pol IV promotes adaptive mutations that are –1 deletions at a variety of mononucleotide repeats (Figure 5), similar to the frameshift component of the error spectrum of the purified enzyme (Wagner et al., 1999). Further, pol IV can account for the requirement for SOS induction in the *lac* system (Figure 2B, Cairns and Foster, 1991; McKenzie et al., 2000). Finally, pol IV is not required for

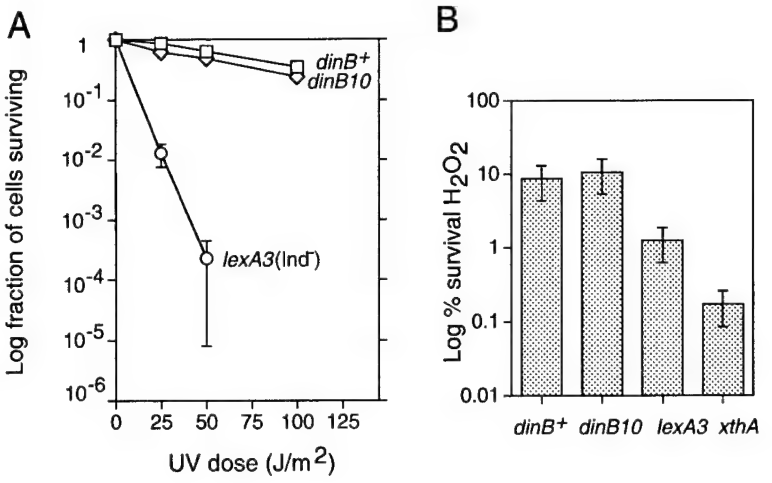


Figure 4. Loss of Pol IV Confers No Detectable Change in Survival of UV or Oxidative Damage

(A) UV sensitivity. Four cultures per strain were tested, and the means ± SEM (error bars) are shown. *DinB*$^+$ (open squares), *dinB10* (open diamonds), and *lexA3*(Ind$^-$) (open circles), strains SMR4562, SMR5830, and FC231, respectively.

(B) Sensitivity to hydrogen peroxide. Four cultures of each strain were tested in parallel, and the mean ± SEM are shown. Strains are as in (A) with the addition of SMR5287 lacking exonuclease III (encoded by *xthA*), used in base excision repair of oxidatively damage DNA (reviewed by Friedberg et al., 1995). Both experiments were performed three times with similar results.

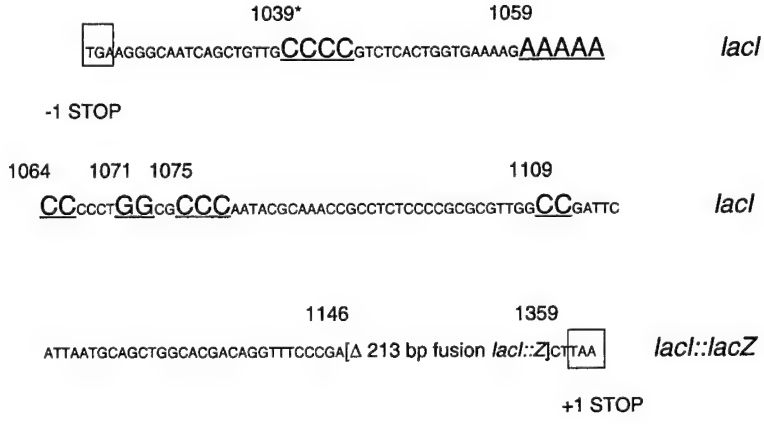
resistance to UV light (Kenyon and Walker, 1980; Figure 4) or hydrogen peroxide (Figure 4).

Adaptive Amplification

The results also reveal that neither pol IV nor induction of SOS/LexA-controlled genes is required for adaptive amplification of *lac* (Figures 1 and 2A). These data add to the evidence that these two adaptive mechanisms are distinct by showing that they require different proteins. These data also suggest that the role of pol IV (and SOS induction) is in error-prone DNA synthesis that generates adaptive point mutations, but not generally in DNA synthesis in stationary phase, which would be expected to be required for both amplification and point mutation mechanisms.

Contributions of Pol IV and MMR Limitation to Mutability and the Characteristic Sequences of *lac* Adaptive Point Mutations

The requirement for an error-prone polymerase, pol IV, in adaptive point mutation supports models in which special error-prone synthesis leads to mutation, making previous models invoking depressed mismatch repair (MMR) as the sole basis of mutability implausible. However, limiting MMR also appears to contribute. First, apart from resembling the frameshift errors made by pol



Mutation	*dinB*$^+$	*dinB10*
-1 at hotspot mononucleotide repeat: nt 1039	22	24
-1 at other mononucleotide repeats	13	1
Other insertions and deletions	0	6
Total:	35	31

Figure 5. DNA Pol IV Promotes –1 Deletions at a Variety of Mononucleotide Repeat Sites in Lac$^+$ Adaptive Mutation

A roughly 300 nucleotide (nt) segment of DNA spanning the *lac* frameshift allele was sequenced from PCR-amplified DNA from day 5 *dinB10* Lac$^+$ point mutants (primers lacIL2 5′–AGGCTATTCTGGTGGCCGGA, and lacD2-GCCTCTTCGCTATTACGCCAGCT). Sequencing was performed by Lone Star Labs, Inc. (Houston, TX). Compensatory frameshift mutations in a possible 130 nt region between the two out-of-frame stop codons (boxed) can restore gene function. In *dinB*$^+$ cells, adaptive reversions are –1 deletions at a hot spot (nt 1039) and at many different mononucleotide repeats sites highlighted above (nt 1059, 1064, 1071, 1075, and 1109, data from Rosenberg et al. 1994). In *dinB10* cells, only the hot spot repeat is appreciably active for –1 repeat deletions, and other insertions and deletions are also prevalent. The other mutations include a –1 frameshift with an adjacent substitution (at nt 1094–5); a +2 insertion (nt 1092); an insertion of >40 bp (from 3′ of the sequenced area to nt 1120); and three large deletions of 103 bp (nt 1017–1119), 103 bp (979–1081), and 211 bp (nt 878–1088). Nt repeat positions are indicated above the leftmost base covered by the number, and the additional base of the original +1 frameshift mutation in the repeat at nt 1039 is not numbered.

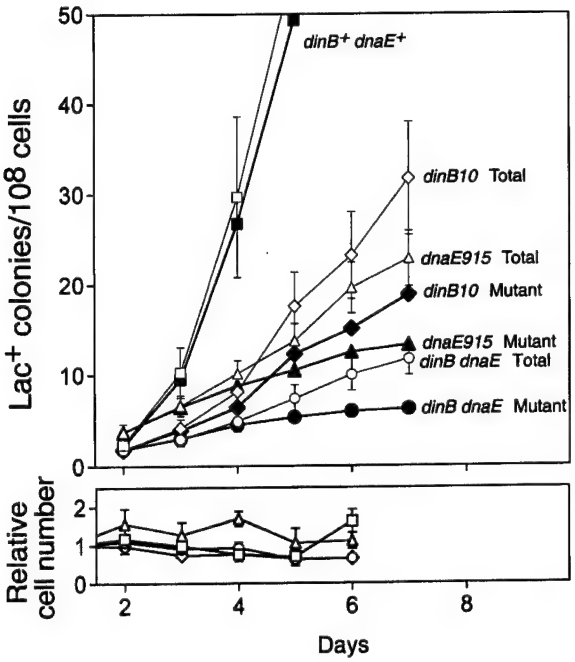


Figure 6. Overlapping Roles of Pol III and Pol IV in Adaptive Point Mutation

Open symbols are total Lac$^+$ colonies, and filled symbols point mutants only for strains carrying *dinB*$^+$ *dnaE*$^+$ (squares), *dinB10* (diamonds), *dnaE915* (triangles), and *dinB10 dnaE915* (circles): SMR6113, SMR5945, SMR6114, and SMR5944, respectively. The experiment was performed three times with similar results. Means ± SEM (error bars) of ten independent cultures tested are shown. Where not visible, error bars are smaller than the plot symbol. Daily measurements of viable *lac*$^-$ cells on the plates (Relative cell number), shown normalized to the first day's count, show no net growth or death during the experiments (mean ± SEM, four cultures).

IV enzyme, the Lac$^+$ adaptive mutation sequences are identical to growth-dependent mutations in cells lacking postsynthesis MMR (Longerich et al., 1995). Second, MMR limitation has been demonstrated to occur, and to be required for, efficient adaptive mutation in this system (Harris et al., 1997b, 1999). Error-prone synthesis and limiting MMR are therefore both implicated and might possibly be related. For example, Wagner and Nohmi (2000) report that pol IV overproduction causes an insufficiency of MMR activity that can be alleviated by overproducing MutL. MutL also becomes limiting for MMR during adaptive mutation (Harris et al., 1997b, 1999) and in mutants with an error-prone DNA polymerase III (Schaaper and Radman, 1989). In all these cases, it could be that excess polymerase errors titrate MMR, causing a synergistic hypermutable condition. However, for Kim et al. (1997), pol IV overproduction did not produce a mutation spectrum similar to that of MMR mutant cells. This implies that MMR was not limiting in their overproduction experiments. Whether the demonstrated MMR limitation during adaptive mutation (Harris et al., 1997b, 1999) is caused by, or independently of, pol IV-produced errors, the combination is likely to interact synergistically to produce a condition of hypermutation.

Roles of Other DNA Polymerases

E. coli has five DNA polymerases. Pols II, III, and IV have been implicated in the synthesis during adaptive mutation as follows. First, the error-free SOS DNA pol II appears to compete with the polymerase(s) making adaptive mutations, in that pol II-deficiency increases adaptive mutation (Foster et al., 1995; Harris, 1997). Perhaps pol II competes with pol IV at the replisome. Second, an anti-mutator pol III allele decreases Lac$^+$ adaptive mutation ≥3-fold (Foster et al., 1995; Harris et al., 1997a), decreasing both pol IV-dependent and pol IV-independent point mutation (Figure 6). The apparent overlap between pol III and pol IV (Figure 6) can be understood by hypotheses in which pol III and pol IV compete with and/or substitute for each other on DNA (e.g., Friedberg et al., 2000; Tang et al., 2000). In one general model, pol IV makes the errors that become mutations. This is supported by the similarity of the sequence spectrum of adaptive mutations attributable to pol IV (Figure 5) with the frameshift error spectrum of the purified polymerase (Wagner et al. 1999). The pol III antimutator protein might exclude pol IV from DNA (and might then lower pol IV-independent point mutations by excluding some other polymerase). Alternatively, pol III might correct errors made by pol IV. It could also do both. In another general model, pol III could make errors that are fixed as mutations by pol IV (see Tang, et al. 2000). Other hypotheses are also possible. Whichever may be the case, the data indicate involvement of both polymerases and suggest that replisomes may exchange pols II (see above), III, and IV.

Function of Pol IV for *E. coli*

A biological function can now be assigned to pol IV, a member of the DinB branch of DinB/UmuDC superfamily polymerases, in adaptive mutation. Is this its only function? Other polymerases in the UmuDC, Rad30, and Rev1 branches of this superfamily are translesion polymerases (Friedberg et al., 2000), but the evidence for pol IV is ambiguous. Purified pol IV deals poorly with common UV lesions (Tang, et al. 2000), and pol IV-defective cells are not sensitive to UV (Kenyon and Walker, 1980, and Figure 4A) or hydrogen peroxide (Figure 4B). Although, together with pol V, pol IV was implicated in synthesis across benzo(*a*)pyrene adducts (Napolitano et al., 2000), that study used a deletion of *dinB* and part of *yafN* (probably also polar on *yafO* and *yafP*, see Experimental Strategy), making the conclusion uncertain. If translesion synthesis at adducts truly is a function of pol IV, it is a different role than the one pol IV plays in adaptive mutation because the former requires pol V (Napolitano et al., 2000), whereas the latter is pol V independent (Cairns and Foster, 1991; McKenzie et al., 2000). Pol IV might facilitate DNA replication promoted by DSBR recombination, the proposed source of replication in adaptive mutation (Harris et al., 1994). Yeast Rev3, or pol zeta (Rev1 subfamily), promotes substitution mutations associated with yeast DSBR (Holbeck and Strathern, 1997). Regardless of other possible functions of pol IV, its central role in adaptive mutability recalls suggestions of enzymes specialized for mutability (Radman, 1975; Echols, 1981, and others subsequently), accelerating evolution when needed.

Role of This Adaptive Mutation Mechanism in Bacterial Evolution

Frameshift mutations are usually thought of as inactivating genes. Is recombination-dependent adaptive muta-

tion generally relevant to bacterial evolution? First, in adaptive mutation at *lac*, substitutions probably also occur because overproduction of pol IV causes substitutions as well as frameshifts (Kim et al., 1997; Wagner and Nohmi, 2000). Second, many pathogenic bacteria regulate expression of "contingency genes" (used under stress) by frequent frameshift mutation events that turn gene functions off and on (e.g., Deitsch et al., 1997; Saunders et al., 2000). These bacteria might employ adaptive mutation strategies similar to those discussed here. In fact, the pathogens *Neisseria meningitidis* and *N. gonorrhoeae* have one or more genes homologous to *dinB* (open reading frame NMB1448 in strain MC58, Tettelin et al., 2000; and NMA1661 in strain Z2491, Parkhill et al., 2000). Third, regarding the relative importance of inducible mutation mechanisms, versus selection of preexisting mutator strains, we note that the mutator strains found among wild bacteria represent the minority (LeClerc et al., 1996; Matic et al., 1997; Denamur et al., 2000; Oliver et al., 2000). The majority of wild bacteria (80%–99%) are not mutators, such that adaptive mutation strategies may contribute appreciably (Rosenberg et al., 1998; Hastings et al., 2000).

Eukaryotic Homologs

Pol IV promotes inducible genetic change (above). Could its mammalian homologs function similarly? The mouse pol IV homologs, pol μ and pol λ, and true ortholog, DinB1 or pol κ (each also in humans), are abundant in lymphoid (μ) and germline cells (λ and κ), respectively (Friedberg et al., 2000). Their functions are unknown, although roles in somatic hypermutation (Friedberg et al., 2000) or other generation of diversity in immunoglobulin and/or T cell receptor genes seem possible. Could there be programmed mutation, driving evolution, in germ cells of mammals? As with the immune system, selections against deleterious mutations are stringent in germ cells (successful completion of development) such that programmed germline mutation/evolution might not be impossible.

Experimental Procedures

Bacterial Strains and Mutant Alleles

Bacterial strains used are isogenic to FC40 (Cairns and Foster, 1991, see also for FC231) and were constructed using standard P1 transduction methods (Miller, 1992). *dinB10* (Wagner et al., 1999) was constructed by PCR site-directed mutagenesis, replaced in the chromosome (Link et al., 1997) and transduced into a *proAB*$^+$ strain to link it with *proAB*$^+$. *proAB*$^+$ *dinB10* was transduced into the F′ replacing *proAB-81*::Tn*10*. The F$^-$ parent of FC40 (Cairns and Foster, 1991) was also transduced to carry *dinB10*, then mated with the F′ *lac* carrying *dinB10* to make the *dinB10* homozygous strain, SMR5830. *dinB10* was identified by (positive) DraI digestion of PCR products. Ectopic expression of *dinB*$^+$ in SMR5834 and SMR5851 was accomplished by replacement of the bacterial *attB* site with *dinB*$^+$ including its natural promoter (basepairs 249,092–255,436 of the *E. coli* genome sequence, as described; L. Gumbiner-Russo, M.-J. Lombardo, and S. M. Rosenberg, unpublished data). SMR583 (FC40 *malB*::Tn*9*), SMR820 (FC40 *malB*::Tn*9* *lexA3*(Ind$^-$)), SMR5849 (SMR5830 *malB*::Tn*9*), and SMR5850 (SMR5830 *malB*::Tn*9*/*lexA3*(Ind$^-$)) carry *malB*::Tn*9* from D. Ennis (Lafayette, LA) and *lexA3*(Ind$^-$) from FC231 (Cairns and Foster, 1991). SMR5287 carries Δ(*xthA-pncA*)*90 zdi-201*::Tn*10* from BW9116 (*E. coli* Genetic Stock Center, Yale University). SMR6113 (FC40 *zae*::Tn*10dcam zae-502*::Tn*10*), SMR6114 (FC40 *zae*::Tn*10dcam dnaE915 zae-502*::Tn*10*), SMR5944 (SMR5830 *zae*::Tn*10dcam dnaE915 zae-502*::Tn*10*), and SMR5945 (SMR5830 *zae*::Tn*10dcam zae-502*::Tn*10*) carry alleles from NR9915 and NR9918 (Fijalkowska et al., 1993). SMR4576 and SMR6049 carrying *upp*::Tn*10dtet+1* (with a 4G to 5G frameshift at bp 331 of *tetA*; Foster, 1997) are described by H. J. Bull, M.-J. Lombardo, and S. M. Rosenberg (unpublished data).

Mutation and Amplification Assays

Adaptive mutation experiments were performed as described (Harris et al., 1996). Daily measurements of viable *lac*$^-$ cells on the plates (Harris et al., 1996) showed no net growth or death during the experiments. Growth-dependent Lac$^+$ mutation measurements used 40 tube fluctuation tests, as described (Harris et al., 1999). Mutation rates were calculated by the method of the median (Lea and Coulson, 1949; as modified by von Borstel, 1978). Other mutations rate assays used 30 tube fluctuation tests with Tet^R, Val^R, and Nal^R calculated by the method of the median and Strep^R and Spec^R by the P_0 method (Lea and Coulson, 1949; von Borstel, 1978; correction for P_0 as per Rosche and Foster, 2000). Because Tet^R colonies continue to appear over time, Tet^R assays were done with Tet^R controls as described for Lac (Harris et al., 1999, Results), to exclude mutants formed on the Tet plates and were scored at 12 hr (90%–100% of the control colonies visible). Selection agents were tetracycline, 10 μg/ml; valine, 5 μg/ml; streptomycin, 100 μg/ml; spectinomycin, 100 μg/ml; and nalidixic acid, 10 μg/ml.

The fraction of Lac$^+$ colonies carrying amplification rather than point mutation was determined in *dinB*$^+$ and *dinB10* day 5 Lac$^+$ colonies (40 colonies/culture, four independent cultures) of each strain as previously described (Hastings et al., 2000) by picking and restreaking Lac$^+$ colonies to LBH X-gal rifampicin medium to test instability of the Lac$^+$ phenotype. Unstable Lac$^+$ carry roughly 30 copies of *lac*$^-$ amplified DNA in direct repeats of 7–40 kb (Hastings et al., 2000). This method was also used for Figures 2 and 6.

UV and Oxidative Damage Survival Assays

Diluted saturated cultures (four/strain) in LBH medium (e.g., Torkelson et al., 1997) were plated on LBH plates and irradiated in a Stratalinker (Stratagene, La Jolla, CA). Sensitivity to hydrogen peroxide (H_20_2) was measured as described (Demple et al., 1983), splitting log phase LBH cultures, exposing half to 5.6 mM H_2O_2 (and half to H_20_2-free control medium) for 15 min, and plating for viable cells.

Acknowledgments

We thank R.S. Harris for identifying the merodiploidy of *dinB*, G. Church for plasmid pKO3, H. Ohmori for discussion, our friend M. Radman for discussion of our results on this study over the past 4 years, A. Beaudet, H.J. Bull, E. Friedberg, P. Hanawalt, J. Lupski, and D. Nelson for comments on the manuscript, and M. Price, I. Aponyi, and J. Ross for technical assistance. We acknowledge B.A. Roe, L. Song, S. P. Lin, X. Yuan, S. Clifton, T. Ducey, L. Lewis, D.W. Dyer, and the Gonococcal Genome Sequencing Project supported by National Institutes of Health (NIH) AI38399. This work was supported by a Department of Defense Breast Cancer Research Graduate Fellowship (G. J. M.) and NIH grants F32-GM19909 (to M.-J. L.), R01-GM53158, and R01-AI43917.

Received December 6, 2000; revised February 26, 2001.

References

Brotcorne-Lannoye, A., and Maenhaut-Michel, G. (1986). Role of RecA protein in untargeted UV mutagenesis of bacteriophage lambda: evidence for the requirement for the *dinB* gene. Proc. Natl. Acad. Sci. USA *83*, 3904–3908.

Bull, H.J., McKenzie, G.J., Hastings, P.J., and Rosenberg, S.M. (2000a). Evidence that stationary-phase hypermutation in the *Escherichia coli* chromosome is promoted by recombination. Genetics *154*, 1427–1437.

Bull, H.J., McKenzie, G.J., Hastings, P.J., and Rosenberg, S.M. (2000b). The contribution of transiently hypermutable cells to mutation in stationary phase. Genetics *156*, 925–926.

Cairns, J. (2000). The contribution of bacterial hypermutators to mutation in stationary phase. Genetics *156*, 923.

Cairns, J., and Foster, P.L. (1991). Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics *128*, 695–701.

Courcelle, J., Khodursky, A., Peter, B., Brown, P.O., and Hanawalt, P.C. (2001). Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics, in press.

Deitsch, K.W., Moxon, E.R., and Wellems, T.E. (1997). Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. Microbiol. Mol. Biol. Rev. *61*, 281–293.

Demple, B., Halbrook, J., and Linn, S. (1983). *Escherichia coli xth* mutants are hypersensitive to hydrogen peroxide. J. Bacteriol. *153*, 1079–1082.

Denamur, E., Lecointre, G., Darlu, P., Tenaillon, O., Acquaviva, C., Sayada, C., Sunjevaric, I., Rothstein, R., Elion, J., Taddei, F., et al. (2000). Evolutionary implications of the frequent horizontal transfer of mismatch repair genes. Cell *103*, 711–721.

Echols, H. (1981). SOS functions, cancer and inducible evolution. Cell *25*, 1–2.

Fijalkowska, I.J., Dunn, R.L., and Schaaper, R.M. (1993). Mutants of *Escherichia coli* with increased fidelity of DNA replication. Genetics *134*, 1023–1030.

Foster, P.L. (1997). Nonadaptive mutations occur in the F′ episome during adaptive mutation conditions in *Escherichia coli*. J. Bacteriol. *179*, 1550–1554.

Foster, P.L. (1999). Mechanisms of stationary phase mutation: a decade of adaptive mutation. Annu. Rev. Genet. *33*, 57–88.

Foster, P.L., and Trimarchi, J.M. (1994). Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. Science *265*, 407–409.

Foster, P.L., Gudmundsson, G., Trimarchi, J.M., Cai, H., and Goodman, M.F. (1995). Proofreading-defective DNA polymerase II increases adaptive mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA *92*, 7951–7955.

Foster, P.L., Trimarchi, J.M., and Maurer, R.A. (1996). Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. Genetics *142*, 25–37.

Friedberg, E.C., Walker, G.C., and Siede, W. (1995). DNA Repair and Mutagenesis (Washington, D.C.: ASM Press).

Friedberg, E.C., Feaver, W.J., and Gerlach, V.L. (2000). The many faces of DNA polymerases: strategies for mutagenesis and for mutational avoidance. Proc. Natl. Acad. Sci. USA *97*, 5681–5683.

Godoy, V.G., Gizatullin, F.S., and Fox, M.S. (2000). Some features of the mutability of bacteria during nonlethal selection. Genetics *154*, 49–59.

Grønlund, H., and Gerdes, K. (1999). Toxin-antitoxin systems homologous with *relBE* of *Escherichia coli* plasmid P307 are ubiquitous in prokaryotes. J. Mol. Biol. *285*, 1401–1415.

Harris, R.S. (1997). On a molecular mechanism of adaptive mutation. Ph.D. thesis (Edmonton: University of Alberta).

Harris, R.S., Longerich, S., and Rosenberg, S.M. (1994). Recombination in adaptive mutation. Science *264*, 258–260.

Harris, R.S., Ross, K.J., and Rosenberg, S.M. (1996). Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. Genetics *142*, 681–691.

Harris, R.S., Bull, H.J., and Rosenberg, S.M. (1997a). A direct role for DNA polymerase III in adaptive reversion of a frameshift mutation in *Escherichia coli*. Mutat. Res. *375*, 19–24.

Harris, R.S., Feng, G., Ross, K.J., Sidhu, R., Thulin, C., Longerich, S., Szigety, S.K., Winkler, M.E., and Rosenberg, S.M. (1997b). Mismatch repair protein MutL becomes limiting during stationary-phase mutation. Genes Dev. *11*, 2426–2437.

Harris, R.S., Feng, G., Ross, K.J., Sidhu, R., Thulin, C., Longerich, S., Szigety, S.K., Hastings, P.J., Winkler, M.E., and Rosenberg, S.M. (1999). Mismatch repair is diminished during stationary-phase mutation. Mutat. Res. *437*, 51–60.

Hastings, P.J., Bull, H.J., Klump, J.R., and Rosenberg, S.M. (2000). Adaptive amplification: an inducible chromosomal instability mechanism. Cell *103*, 723–731.

Holbeck, S.L., and Strathern, J.N. (1997). A role for REV3 in mutagenesis during double-strand break repair in *Saccharomyces cerevisiae*. Genetics *147*, 1017–1024.

Kenyon, C.J., and Walker, G.C. (1980). DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. USA *77*, 2819–2823.

Kim, S.R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., Nohmi, T., and Ohmori, H. (1997). Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an overexpression of *dinB/dinP* results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. Proc. Natl. Acad. Sci. USA *94*, 13792–13797.

LaRossa, R.A. (1996). Mutant selections linking physiology, inhibitors, and genotypes. In *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, eds. (Washington, D.C.: ASM Press), pp. 2527–2587.

Lea, D.E., and Coulson, C.A. (1949). The distribution of the numbers of mutants in bacterial populations. J. Genet. *49*, 264–285.

LeClerc, J.E., Li, B., Payne, W.L., and Cebula, T.A. (1996). High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science *274*, 1208–1211.

Lin, L.L., and Little, J.W. (1989). Autodigestion and RecA-dependent cleavage of Ind- mutant LexA proteins. J. Mol. Biol. *210*, 439–452.

Link, A.J., Phillips, D., and Church, G.M. (1997). Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: application to open reading frame characterization. J. Bacteriol. *179*, 6228–6237.

Longerich, S., Galloway, A.M., Harris, R.S., Wong, C., and Rosenberg, S.M. (1995). Adaptive mutation sequences reproduced by mismatch repair deficiency. Proc. Natl. Acad. Sci. USA *92*, 12017–12020.

Matic, I., Radman, M., Taddei, F., Picard, B., Doit, C., Bingen, E., Denamur, E., and Elion, J. (1997). Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. Science *277*, 1833–1834.

McKenzie, G.J., Lombardo, M.-J., and Rosenberg, S.M. (1998). Recombination-dependent mutation in *Escherichia coli* occurs in stationary phase. Genetics *149*, 1163–1165.

McKenzie, G.J., Harris, R.S., Lee, P.L., and Rosenberg, S.M. (2000). The SOS response regulates adaptive mutation. Proc. Natl. Acad. Sci. USA *97*, 6646–6651.

Miller, J.H. (1992). A Short Course in Bacterial Genetics (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Modrich, P., and Lahue, R. (1996). Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. *65*, 101–133.

Mount, D.W., Low, K.B., and Edmiston, S.J. (1972). Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. *112*, 886–893.

Napolitano, R., Janel-Bintz, R., Wagner, J., and Fuchs, R.P.P. (2000). All three SOS-inducible DNA polymerases (pol II, pol IV, and pol V) are involved in induced mutagenesis. EMBO J. *19*, 6259–6265.

Oliver, A., Cantón, R., Campo, P., Baquero, F., and Blázquez, J. (2000). High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science *288*, 1251–1254.

Parkhill, J., Achtman, M., James, K.D., Bentley, S.D., Churcher, C., Klee, S.R., Morelli, G., Basham, D., Brown, D., Chillingworth, T., et al. (2000). Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. Nature *404*, 502–506.

Radman, M. (1975). SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis. Basic Life Sci. *5A*, 355–367.

Ripley, L.S. (1990). Frameshift mutation: determinants of specificity. Annu. Rev. Genet. *24*, 189–213.

Rosche, W.A., and Foster, P.L. (1999). The role of transient hypermu-

tators in adaptive mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA *96*, 6862–6867.

Rosche, W.A., and Foster, P.L. (2000). Determining mutation rates in bacterial populations. Methods *20*, 4–17.

Rosenberg, S.M. (1997). Mutation for survival. Curr. Opin. Genet. Dev. *7*, 829–834.

Rosenberg, S.M. (2001). Evolution at will: adaptive mutation. Nat. Rev. Genet., in press.

Rosenberg, S.M., Longerich, S., Gee, P., and Harris, R.S. (1994). Adaptive mutation by deletions in small mononucleotide repeats. Science *265*, 405–407.

Rosenberg, S.M., Thulin, C., and Harris, R.S. (1998). Transient and heritable mutators in adaptive evolution in the lab and in nature. Genetics *148*, 1559–1566.

Saunders, N.J., Jeffries, A.C., Peden, J.F., Hood, D.W., Tettelin, H., Rappuoli, R., and Moxon, E.R. (2000). Repeat-associated phase variable genes in the complete genome sequence of *Neisseria meningitidis* strain MC58. Mol. Microbiol. *37*, 207–215.

Schaaper, R.M., and Radman, M. (1989). The extreme mutator effect of *Escherichia coli mutD5* results from saturation of mismatch repair by excessive DNA replication errors. EMBO J. *8*, 3511–3516.

Strauss, B.S., Roberts, R., Francis, L., and Pouryazdanparast, P. (2000). Role of the *dinB* gene product in spontaneous mutation in *Escherichia coli* with an impaired replicative polymerase. J. Bacteriol. *182*, 6742–6750.

Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., and Inouye, M. (1966). Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol. *31*, 77–84.

Tang, M., Pham, P., Shen, X., Taylor, J.S., O'Donnell, M., Woodgate, R., and Goodman, M.F. (2000). Roles of *E. coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis. Nature *404*, 1014–1018.

Tettelin, H., Saunders, N.J., Heidelberg, J., Jeffries, A.C., Nelson, K.E., Eisen, J.A., Ketchum, K.A., Hood, D.W., Peden, J.F., Dodson, R.J., et al. (2000). Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. Science *287*, 1809–1815.

Torkelson, J., Harris, R.S., Lombardo, M.-J., Nagendran, J., Thulin, C., and Rosenberg, S.M. (1997). Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. EMBO J. *16*, 3303–3311.

von Borstel, R.C. (1978). Measuring spontaneous mutation rates in yeast. Methods Cell Biol. *20*, 1–24.

Wagner, J., and Nohmi, T. (2000). *Escherichia coli* DNA polymerase IV mutator activity: genetic requirements and mutational specificity. J. Bacteriol. *182*, 4587–4595.

Wagner, J., Gruz, P., Kim, S.R., Yamada, M., Matsui, K., Fuchs, R.P., and Nohmi, T. (1999). The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis. Mol. Cell *4*, 281–286.

Wagner, J., Fujii, S., Gruz, P., Nohmi, T., and Fuchs, R.P.P. (2000). The β clamp targets DNA polymerase IV to DNA and strongly increases its processivity. EMBO Reports *1*, 484–488.

Walker, G.C. (1996). The SOS response of *Escherichia coli*. In *Escherichia coli* and *Salmonella:* Cellular and Molecular Biology, F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, eds. (Washington, D.C.: ASM Press), pp. 1400–1416.

Molecular Cell, Vol. 7, 1119, May, 2001, Copyright ©2001 by Cell Press

Erratum

In the March *Molecular Cell* article by McKenzie et al., "SOS Mutator DNA Polymerase IV Functions in Adaptive Mutation and Not Adaptive Amplification" (*7*, 571–579), Table 1 contained four incorrect numbers in the column "Growth-Dependent Mutation Rate to Lac$^+$ (Mutations/Cell/Generation)". The mean mutation rates in the table were correct. The conclusions from this table and of the paper are not altered by this correction. The corrected Table 1 is printed below and will be corrected in the online version of the article.

Table 1. DNA Polymerase IV Does Not Affect *lac* Frameshift Reversion in Growing Cells

Relevant Genotype	Experiment	Median Number of Mutants	Growth-Dependent Mutation Rate to Lac$^+$ (Mutations/Cell/Generation)	Mean (± SEM)
dinB$^+$	1	3.5	0.96×10^{-9}	$1.6 (\pm 0.3) \times 10^{-9}$
	2	12.8	2.3×10^{-9}	
	3	5.1	1.5×10^{-9}	
	4	5.0	1.8×10^{-9}	
dinB10	1	2.0	0.63×10^{-9}	$1.2 (\pm 0.3) \times 10^{-9}$
	2	7.4	1.9×10^{-9}	
	3	2.9	1.3×10^{-9}	
	4	3.0	1.1×10^{-9}	

Strains are *dinB*$^+$, SMR4562 and *dinB10*, SMR5830. See Experimental Procedures in the article.

The SOS response regulates adaptive mutation

Gregory J. McKenzie[†], Reuben S. Harris[‡§], Peter L. Lee[†], and Susan M. Rosenberg[†‡¶]

[†]Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; and [‡]Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Communicated by Evelyn M. Witkin, Rutgers, The State University of New Jersey at New Brunswick, Princeton, NJ, April 10, 2000
(received for review February 8, 2000)

Upon starvation some *Escherichia coli* cells undergo a transient, genome-wide hypermutation (called adaptive mutation) that is recombination-dependent and appears to be a response to a stressful environment. Adaptive mutation may reflect an inducible mechanism that generates genetic variability in times of stress. Previously, however, the regulatory components and signal transduction pathways controlling adaptive mutation were unknown. Here we show that adaptive mutation is regulated by the SOS response, a complex, graded response to DNA damage that includes induction of gene products blocking cell division and promoting mutation, recombination, and DNA repair. We find that SOS-induced levels of proteins other than RecA are needed for adaptive mutation. We report a requirement of RecF for efficient adaptive mutation and provide evidence that the role of RecF in mutation is to allow SOS induction. We also report the discovery of an SOS-controlled inhibitor of adaptive mutation, PsiB. These results indicate that adaptive mutation is a tightly regulated response, controlled both positively and negatively by the SOS system.

DNA repair | *Escherichia coli* | signal transduction | RecF | RecA

The bacterial SOS response, studied extensively in *Escherichia coli*, is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis are induced (1). SOS is the prototypic cell cycle check-point control and DNA repair system, and because of this, a detailed picture of the signal transduction pathway that regulates this response is understood. A central part of the SOS response is the de-repression of more than 20 genes under the direct and indirect transcriptional control of the LexA repressor. The LexA regulon includes recombination and repair genes *recA*, *recN*, and *ruvAB*, nucleotide excision repair genes *uvrAB* and *uvrD*, the error-prone DNA polymerase (pol) genes *dinB* (encoding pol IV) (2) and *umuDC* (encoding pol V) (3), and DNA polymerase II (4, 5) in addition to many functions not yet understood. In the absence of a functional SOS response, cells are sensitive to DNA damaging agents.

The signal transduction pathway leading to an SOS response (reviewed by ref. 6) ensues when RecA protein binds to single-stranded DNA (ssDNA), which can be created by processing of DNA damage, stalled replication, and perhaps by other means (7–9). The ssDNA acts as a signal that activates an otherwise dormant co-protease activity of RecA, which allows activated RecA (called RecA*) to facilitate the proteolytic self-cleavage of the LexA repressor, thus inducing the LexA regulon (10). Activated RecA also facilitates the cleavage of phage repressors used to maintain the quiescent, lysogenic state, and UmuD, creating UmuD′, the subunit of UmuD′C (pol V) that allows activity in trans-lesion error-prone DNA synthesis (6).

An intriguing feature of the SOS response is inducible mutation (11, 12). LexA-repressed pol V participates in most UV mutagenesis, by inserting bases across from pyrimidine dimers (3). Pol IV is required for an indirect mutation phenomenon in which undamaged phage λ DNA is mutated when added to UV-irradiated (SOS-induced) cells (13). There may be other mutagenic mechanisms induced by the SOS response.

Adaptive mutation (also called stationary-phase mutation) is a collection of phenomena in which mutations form in stressed or starving, nongrowing, or slowly growing cells, and at least some of these mutations allow growth (reviewed by refs. 14–19). It is a model for mutational escape of growth-control, such as in oncogenesis, tumor progression, and resistance to chemotherapeutic drugs (16, 20–22), and also, like SOS mutagenesis, implies that evolution can be hastened when the need arises (23).

Adaptive mutation has been studied most extensively using an assay for reversion of a *lac* +1 frameshift allele on an F′ sex plasmid in *E. coli* starved on lactose medium (24). The adaptive mutations are unlike Lac$^+$ mutations in growing cells in that they form during (not before) exposure to selective conditions (25), and occur via a unique molecular mechanism (reviewed by refs. 18 and 19) that requires homologous recombination proteins RecA, RecBC, and RuvABC (22, 26, 27). The adaptive mutations occur in a hypermutable subpopulation of the starved cells (28–30) during a transient period of limiting mismatch-repair activity (31) and possess a unique sequence spectrum of −1 deletions in mononucleotide repeats (32, 33) identical to that of mismatch repair defective cells (34).

As reviewed above, the cells undergoing adaptive mutation are transiently differentiated and mutable. However, the mechanism(s) by which the environment induces this differentiation, the signals from the environment, and the signal transduction pathway(s) provoking adaptive mutation are unknown. We have examined the role of the SOS response in adaptive mutation and report both positive and negative control of adaptive mutation in the Lac system by the LexA repressor. First, we report that SOS induction of the LexA regulon is required for efficient adaptive mutation. Simple overproduction of RecA, a recombination protein controlled by LexA, does not substitute. Second, we provide evidence that RecF protein is required for efficient mutation in its SOS-inducing capacity. This implies that the DNA signal provoking SOS during adaptive mutation is not a DNA double-strand break (DSB) as postulated previously (e.g., ref. 18), and implies that there are ssDNA intermediates in mutation other than at DSBs. Third, we find evidence of an SOS-controlled repressor of adaptive mutation, PsiB, a protein known to inhibit RecA* activity. The adaptive mutation response appears to occur within a narrow window in the continuum of levels of SOS induction. These results (*i*) indicate that adaptive mutation is a tightly regulated response, (*ii*) identify part of the signal transduction pathway that controls it, and (*iii*) illuminate possible DNA intermediates in that signal transduction pathway.

Abbreviations: ssDNA, single-stranded DNA; pol, polymerase; DSB, double-strand break.

[§]Present address: Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH United Kingdom.

[¶]To whom reprint requests should be addressed at: Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room S809A, Mail Stop 225, Houston, TX 77030-3498. E-mail: smr@bcm.tmc.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.120161797. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.120161797

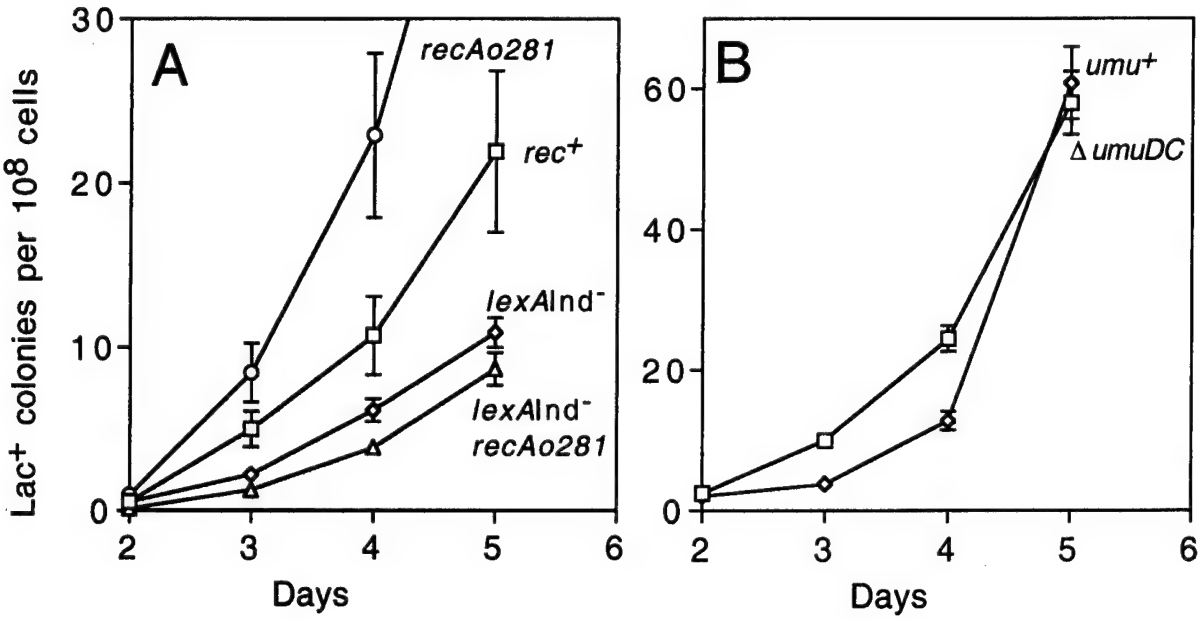


Fig. 1. (*A*) Induction of a LexA-regulated gene(s) other than or in addition to *recA* is required for efficient Lac$^+$ adaptive mutation. ○, *recAo281*; □, *rec*$^+$; ◇, *lexA3(Ind*$^-$*)*; △, *lexA3*(Ind$^-$) *recAo281*. (*B*) *umuDC* is not required for adaptive mutation. □, *Δ(umuDC)595::cat*; ◇, *umu*$^+$. In this and all figures, all strains shown together were tested in parallel, and the means ± SEM (error bars) of several independent cultures tested in parallel are displayed.

Materials and Methods

All new *E. coli* strains were constructed using standard P1 transduction techniques (35). The mutant alleles used were *recAo281* (36), *lexA3*(Ind$^-$) (37), *lexA51*(Def) (38), *sulA211* (*E. coli* Genetic Stock Center, New Haven, CT), *recF332*::Tn*3* (39), *dinI*::*kan* (40), *psiB*::*cat* (A. Bailone, Orsay, France), and *Δ(umuDC)595*::*cat* (41). Strains used in the mutation assay are derived from FC40 (24), which carries a deletion of the chromosomal *lac-pro* region and an F′ carrying *pro*$^+$ and a *lacI33ΩlacZ* fusion with a +1 frameshift mutation such that the cells are phenotypically Lac$^-$. Mutation assays were as described previously (27), including that cell viability measurements for all experiments reported showed no net growth or death of the frameshift-bearing cells. Some variability is seen in absolute values from experiment to experiment, but relative values between strains remained the same within a minimum of three repeats. Single representative experiments are shown (see Figs. 1–3) and the consistency of results across multiple repeats summarized (see Fig. 4).

Results

Induction of a LexA Controlled Gene(s) Other Than or in Addition to RecA Is Required for Adaptive Mutation. The *lexA3*(Ind$^-$) allele encodes a noncleavable mutant LexA protein (42, 43) containing a substitution of Gly-84 to Asp (44, 45). In *lexA3*(Ind$^-$) cells, the LexA regulon is repressed and cannot be induced. In a strain carrying *lexA3*(Ind$^-$), adaptive mutation is decreased 3- to 4-fold (Fig. 1*A*), as seen previously (24). This result indicates a requirement for induced levels of a LexA-repressed gene(s) for efficient adaptive mutation. The LexA-repressed gene(s) could be required absolutely for adaptive mutation if the basal level of expression in uninduced cells is sufficient for some adaptive mutation to occur.

recA is repressed by LexA, and is induced >10-fold during the SOS response (1). RecA is essential for adaptive mutation (22), making it a reasonable candidate for being required at induced levels. To test this hypothesis, we used a *recA* operator-constitutive allele, *recAo281*, that produces induced levels of RecA constitutively (36). In *lexA3*(Ind$^-$) *recAo281* cells, RecA is produced at levels similar to those during SOS induction (36). This allele does not restore the level of adaptive mutation in *lexA3*(Ind$^-$) cells to the level of *lexA*$^+$ cells (Figs. 1*A* and 4*A*), in contrast with data reported previously (24). The strain used by those authors was shown subsequently not to carry *lexA3*(Ind$^-$) (26, 46). This failure to restore mutation with a

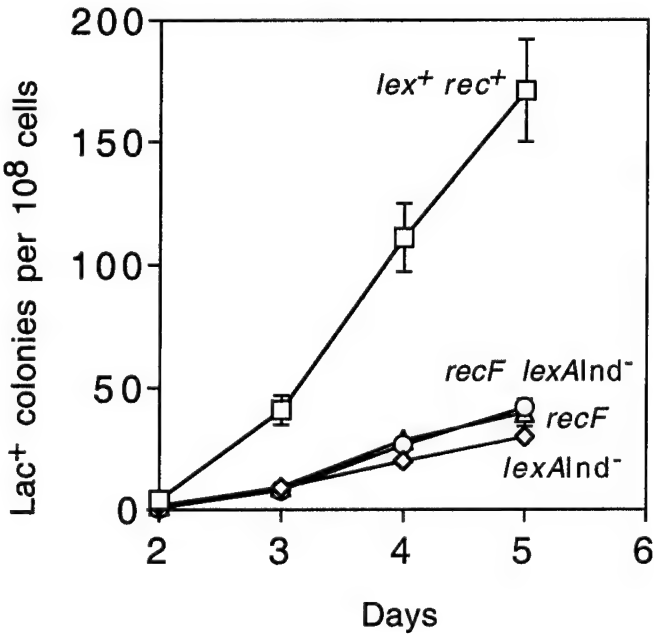
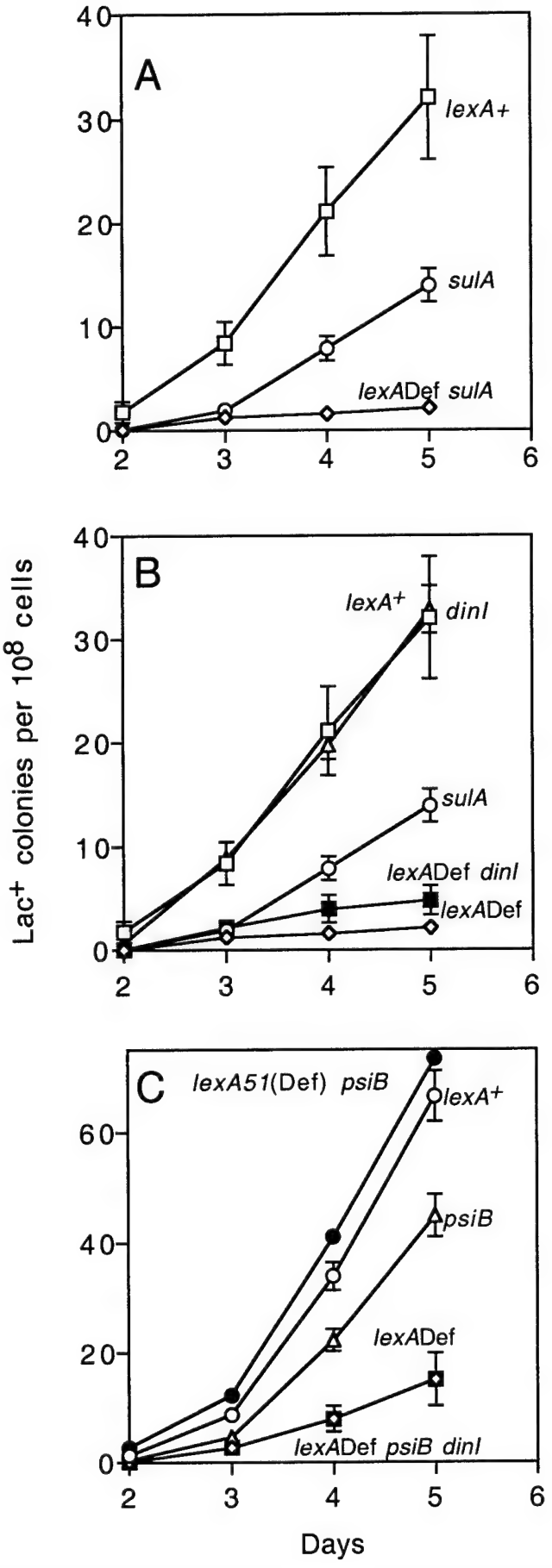


Fig. 2. RecF$^+$ promotes adaptive mutation and acts via the same pathway as LexA. □, *rec*$^+$ *lexA*$^+$; △, *recF332*::Tn*3*; ○, *recF332*::Tn*3 lexA3(Ind*$^-$*)*; ◇, *lexA3(Ind*$^-$*)*.



constitutively expressing *recA* allele indicates that *recA* is either not the LexA-repressed gene, or not the only LexA-repressed gene, required at induced levels for efficient adaptive mutation.

The LexA-repressed function required at induced levels is not the mutagenic UmuDC complex (Fig. 1*B*). This agrees with work showing that a *recA* allele unable to cleave UmuD to the mutagenically active form, UmuD′, does not affect the level of adaptive mutation (24).

RecF Is Required for Efficient Adaptive Mutation. RecF protein plays a poorly defined role(s) in recombination *in vivo* (47, 48). RecF is also required for SOS induction by some DNA damaging agents that produce single-stranded lesions (and not those that produce DSBs) (49). In a *recF* mutant, adaptive mutation is decreased 3- to 5-fold (Fig. 2 and 4*B*). To test whether RecF promotes adaptive mutation via its recombination capacity or via allowing SOS induction, a *recF lexA3*(Ind$^-$) strain was examined. *recF* is epistatic with *lexA3*(Ind$^-$) (Fig. 2), suggesting that the role of RecF in mutation is to allow SOS induction and not via recombination (alternatives discussed below).

A LexA-Controlled Inhibitor of Adaptive Mutation. Because induction of some protein(s) is required, we tested whether constitutive de-repression of the LexA-repressed genes promotes adaptive mutation. Cells lacking LexA must also carry a mutation in the *sulA* gene to be viable because SulA is a LexA-repressed protein that inhibits cell division (50). A *sulA* mutation by itself decreases adaptive mutation slightly (Figs. 3*A* and 4*C*). This could be because more cell division occurs during SOS in the absence of SulA, such that sister chromosomes have more opportunity to segregate and thus less opportunity to recombine. Sister chromosomes are a possible source of the homologous DNA used in the recombination required for adaptive mutation in this system (22). In contrast to the simplest prediction, the *lexA51*(Def) *sulA* cells show greatly decreased adaptive mutation (Fig. 3*A*). This finding indicates that constitutive de-repression of some LexA-repressed gene(s) inhibits adaptive mutation.

We tested two candidates for the LexA-repressed inhibitor(s) of adaptive mutation. DinI is a LexA-repressed protein that inhibits recombination and SOS induction by binding and altering RecA (40). Its proposed function is to help return cells to normal after an SOS response. We find that loss of *dinI* in a *lexA51*(Def) cell has little effect (Figs. 3*B* and 4*C*), indicating that DinI is not an important LexA-repressed inhibitor of adaptive mutation. However, a different anti-SOS protein encoded by the F plasmid, PsiB (51), appears to be the LexA-repressed inhibitor. In the absence of LexA, the loss of PsiB restores adaptive mutation to normal (Figs. 3*C* and 4*C*). PsiB also interacts with RecA to decrease RecA* activity (51). In addition, loss of PsiB in *lexA*$^+$ cells diminishes adaptive mutation. This finding implies that the extent of RecA* activity is crucial to adaptive mutation, indicating a tight regulatory control over adaptive mutation, as does the following result. When *psiB* and *dinI* are both removed in a *lexA51*(Def) strain, adaptive mutation is diminished greatly relative to *psiB lexA51*(Def) (Figs.

Fig. 3. LexA-repressed inhibitor(s) of Lac$^+$ adaptive mutation. (*A*) Complete de-repression of the LexA regulon inhibits mutation. The *lexA* defective strain carries *lexA51*(Def) *sulA211* (◇), *lexA*$^+$ (□), and *sulA211* (○). The *sulA* mutation, required for viability of *lexA51*(Def) strains, also depresses mutation modestly (discussed in text). (*B*) The LexA-controlled inhibitor of adaptive mutation is not DinI. Both *lexA51*(Def) strains also carry *sulA211*. □, *dinI*$^+$ *lexA*$^+$; △, *dinI*::*kan lexA*$^+$; ○, *sulA211*; ■, *lexA51*(Def) *sulA211 dinI*::*kan*; ◇, *lexA51*(Def) *sulA211*. (*C*) PsiB inhibits adaptive mutation in LexA de-repressed cells. All strains shown carry *sulA211*. Additional alleles carried are as follows: ○, *lexA*$^+$; ●, *lexA51*(Def) *psiB*::*cat*; △, *psiB*::*cat*; ◇, *lexA51*(Def); ■, *lexA51*(Def) *psiB*::*cat dinI*::*kan*. Results are discussed in the text.

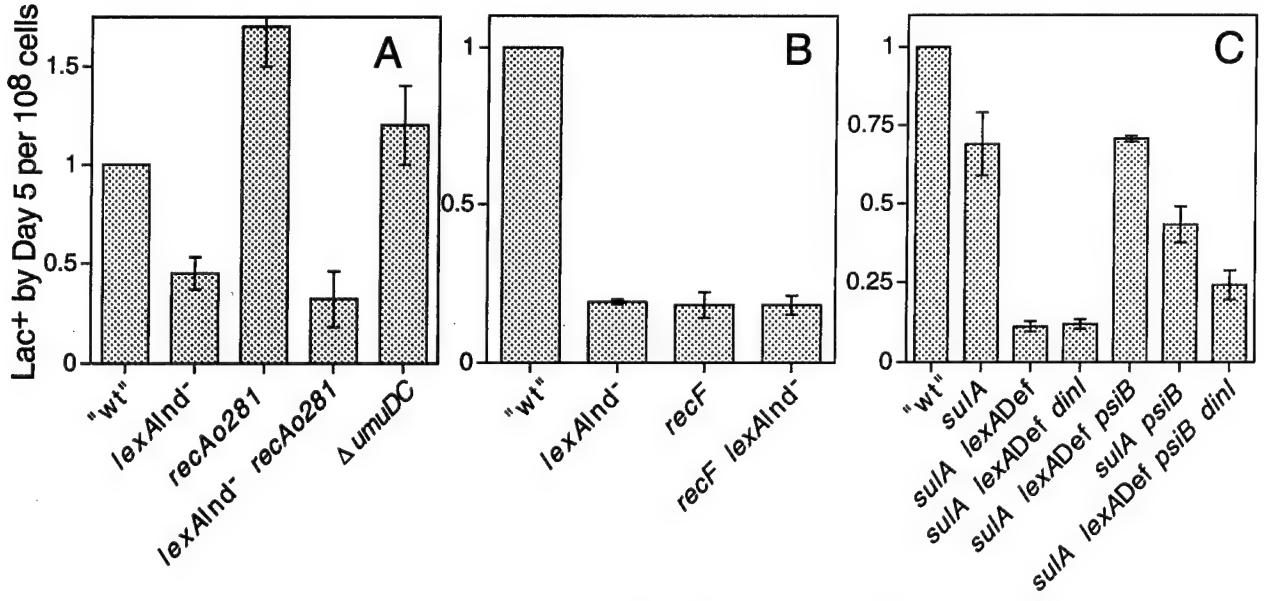


Fig. 4. Comparison of various SOS altered mutants with "wild-type" (wt) in multiple experiments. The fold-differences in accumulation of Lac$^+$ mutant colonies by day 5 is displayed between of each of the mutants listed and the nonmutant parental strain (wt; set equal to 1) assayed in parallel over multiple experiments. The numbers (n) of experiments averaged (error bars equal 1 SEM) are as follows: wt, $n = 4$; *lexA3*(Ind$^-$), $n = 4$; *recAo281*, $n = 4$; *lexA3*(Ind$^-$) *recAo281*, $n = 4$, Δ*umuDC*, $n = 3$ (*A*); $n = 3$ for all genotypes displayed (*B*); and $n = 3$ for all genotypes displayed except "wt" and *sulA lexAdef* for which $n = 4$ (*C*). "wt" is isogenic with all of the mutants used, and carries wild-type alleles of all *rec*, *lex*, *sul*, *din*, and *psi* genes and Δ(*pro-lac*) Rif *thi* F′ *pro*$^+$*laclq lacI33*Ω*lacZ*.

3*C* and 4*C*). This result suggests that *psiB* is not a direct inhibitor of adaptive mutation, but modulates it by modulating the SOS response, and that adaptive mutation is very sensitive to the extent and/or duration of the SOS response, and to levels of RecA* activity. Because RecA* activity is implicated as being important even when the LexA regulon is fully derepressed [in *lexA51*(Def) cells], these results might imply that a target of the RecA* co-protease activity in addition to LexA is important in adaptive mutation (this and alternatives are discussed below).

To summarize: (*i*) PsiB appears to inhibit adaptive mutation when the LexA regulon is constitutively de-repressed in a *lexA*Def mutant; and (*ii*) two proteins that modulate RecA* activity, DinI and PsiB, affect adaptive mutation positively and negatively. These data suggest that RecA* activity is critical in adaptive mutation, that if RecA* activity is either too high or too low, mutation is decreased. These results indicate a tight control over adaptive mutation by factors modulating the SOS response, and provide evidence of SOS regulation of adaptive mutation independent of particular LexA alleles.

Discussion

The results reported indicate that adaptive mutation in the Lac system in *E. coli* is regulated by the SOS system. This identifies SOS as a signal transduction pathway controlling the transient, differentiated condition (52) of adaptive mutation, and likewise identifies adaptive mutation as a new form of SOS mutagenesis.

The LexA Regulon and Adaptive Mutation. We have shown that efficient Lac adaptive mutation requires SOS-induced levels of a LexA-repressed function(s) other than or in addition to RecA (Fig. 1). As discussed above, no real conflict exists between previously reported data (24) and ours.

Two Roles for RecA. RecA is both a signal sensor/transducer molecule for the SOS response and an important recombination protein (53). Because recombination proteins RecBCD, RuvAB, and RuvC are also required for adaptive mutation (22, 26, 27), and RuvAB and RuvC do not affect SOS induction (1), a recombinational role for RecA in adaptive mutation has been clear. The current results indicate that the SOS activation function of RecA is also required for efficient adaptive mutation. These data allow one to understand the previously perplexing finding that a special *recA* mutation conferring recombination-proficiency and SOS deficiency reduces adaptive mutation in this system (24).

DNA Intermediates in Signal Transduction. Efficient SOS induction requires either RecBC or RecF, depending on whether the DNA intermediate that triggers the SOS response is a double-strand end (RecBCD) or ssDNA not at a double-strand end (RecF) (49). RecF is partially required for adaptive mutation (Fig. 2), and the data suggest that this requirement reflects a requirement for RecF in SOS induction during adaptive mutation: (*i*) loss of RecF decreases adaptive mutation to the same (partial) extent as the LexA-uncleavable mutation (Figs. 2 and 4*B*); and (*ii*) RecF deficiency does not reduce mutation further in a strain that is already LexA uncleavable (Figs. 2 and 4*B*), as expected if the sole function of RecF in mutation is to promote LexA cleavage. The converse possibility, that LexA induction is required to produce RecF, is unlikely because RecF is not thought to be LexA regulated (1). Although not ruled out by our data, schemes in which LexA is imagined to function in a RecF-specific recombination route are more complicated, and so are not favored.

The indication that the RecF function in adaptive mutation is to promote the SOS response implies that the ssDNA signal inducing SOS during adaptive mutation is not at a double-strand end (DSE). This is surprising considering that adaptive mutation in this system absolutely requires RecBC (22), an enzyme that operates only at DNA DSEs and breaks (DSBs), and which catalyzes recombinational DSB-repair in *E. coli* by generation of ssDNA at DSEs (54, 55). One possible explanation is that the timing of SOS induction in adaptive mutation necessarily precedes DSB formation. Another is that perhaps, although DSBs form, single-strand lesions are more abundant during adaptive mutation, and so are more important SOS-inducing signals. Whichever is the case, these results allow us to infer a new DNA

intermediate in adaptive mutation: ssDNA other than single-strands exposed at double-strand ends. DSEs (22) and Holliday junctions (26, 27) are the only other DNA intermediates implicated in adaptive mutation, to date.

The ssDNA-inducing SOS during adaptive mutation could be exposed at nicked DNA at the F′ origin of transfer, stalled replication forks or chemically damaged DNA. If nicks at the F′ transfer origin are the signal, this could explain why transfer (Tra) proteins (but not actual transfer) are required for efficient adaptive mutation (56, 57), despite evidence that the F′ need not be covalently linked with the DNA undergoing mutation (28, 58, 59). A *trans* role for the F′ (also suggested by ref. 30), such as inducing *trans*-acting SOS proteins, seems sensible. Further work will be required to determine when, where, and how the ssDNA signal is generated.

Positive and Negative Control. It was surprising to find that in addition to LexA-controlled factor(s) that promote adaptive mutation, there is a LexA-repressed inhibitor, PsiB (Fig. 3). PsiB is a RecA co-protease inhibitor encoded by the F plasmid (51) and may be repressed by LexA (implied by our data, see Fig. 3*C*). The chromosomally encoded DinI protein also blocks RecA co-protease activity and recombination (40). Both of these proteins may promote a speedy return to the non-SOS state after the DNA damage that induced the response has been repaired. The *dinI* deletion had no effect on mutation in either *lexA51*(Def) or *lexA*$^+$ cells, but decreased mutation in the absence of PsiB (Figs. 3*C* and 4*C*). This finding may imply that DinI competes poorly for RecA binding in the presence of PsiB. This apparently perplexing result suggests that levels of RecA* are crucial to successful adaptive mutation. For example, adaptive mutation might be regulated temporally by the SOS response, with both early entry (in LexA-defective cells) and early exit (PsiB$^+$) or late exit (PsiB$^-$ DinI$^-$) from the SOS response being inhibitory to adaptive mutation. Alternatively, cells lacking both PsiB and DinI may simply not survive the SOS induction and hypermutation to form (Lac$^+$) colonies, as follows.

SOS and Hypermutability Are Differentiated States. Recombination-dependent adaptive mutation occurs in a hypermutable subpopulation of the stressed cells (10^{-4} to 10^{-5}) (28, 59). We suggest that SOS induction may be the event that differentiates subpopulation cells from the main population. Although no net cell death was observed during the experiments with the *dinI psiB* strain (see *Materials and Methods*), death of only the subpopulation would have been undetectable.

The discovery that the LexA regulon includes both repressor(s) and promoter(s) of adaptive mutation implies that adaptive mutation is a tightly regulated process. SOS is the first signal transduction pathway found to control adaptive mutation in this system.

Candidate Genes and Molecular Mechanism. The LexA-repressed gene(s) needed at induced levels for efficient Lac-adaptive mutation have not been identified. However, some plausible candidates are suggested by our current picture of the molecular mechanism of adaptive mutation in this system (17–19). The mutations are suggested to result from DNA polymerase errors that occur during the DNA replication (22) now known to be associated with some recombinational double-strand break-repair in *E. coli* (60). The source of the DSBs in the starving cells is not yet known. DSBs may result from stalled replication (22, 61, 62), processing of single-stranded nicks at the F′ transfer origin (63, 64), endonucleases, or chemical damage, or other (e.g., ref. 65). Mismatch repair activity is diminished transiently (28, 34, 52) in the stressed, mutating cells due to a transient limitation of MutL (31, 66). This allows the errors to be fixed as mutations. DNA pol III is implicated in the replication (60, 67, 68). Finally, the mutational process occurs in a small subpopulation of the stressed cells, in which hypermutation occurs at hotspots (not uniformly; ref. 19) throughout the bacterial genome (28–30, 59).

There are several candidate LexA-regulated genes (apart from RecA) whose induction might promote this adaptive mutation mechanism. (*i*) RuvAB recombination proteins (1, 55) are required absolutely for mutation in this system, presumably for the recombination that promotes DNA replication (26, 27). These are expressed constitutively, and may not need to be induced for full recombination (see ref. 69). (*ii*) We found that loss of the SulA cell division inhibitor protein (50, 70) reduces adaptive mutation slightly. Perhaps inhibition of cell division increases the chance of recombination between sister DNA molecules, or lack of division control results in death of some of the subpopulation, which would not be measurable in cell viability determinations. (*iii*) An attractive possibility is the LexA-repressed mutagenic DNA polymerase pol IV, encoded by *dinB* (2, 71). LexA represses three DNA polymerases. Of them, pol II (high accuracy polymerase) inhibits Lac adaptive mutation (46, 67), as if it competes with the mutagenic polymerase that makes the mutations. Pol V (UmuD′C, an error prone polymerase) has no effect (Fig. 1*B*; ref. 24), and pol IV is currently being examined. Pol IV is required for phage λ untargeted mutagenesis (13), and when overexpressed, increases spontaneous mutations (especially −1 frameshifts) up to 800-fold (72). Although DNA pol III is implicated in adaptive mutation (67, 68), the data do not rule out the possibility that another polymerase makes the mutations, or that adaptive mutations are made by both pol III and pol IV (73).

Generality. This report describes the second example of SOS mutagenesis in starving cells independent of UmuDC, both of them dependent on RecA and RecBC. In the first example, aging colonies induce SOS and mutation (74, 75). That SOS response requires cAMP, a signal molecule produced during starvation, and RecB. This is similar to recombination-dependent adaptive mutation (studied here), but the two mutation routes have some different genetic requirements (reviewed by ref. 18) and may represent closely related SOS mutagenesis mechanisms promoted by starvation. UmuDC-dependent SOS transversion mutagenesis in starving cells has also been described (76, 77). Other stationary-phase stress- or starvation-induced mutagenesis mechanisms exist in prokaryotes and eukaryotes (reviewed by refs. 17 and 18), and there are many examples in the literature of recombination-associated mutation in eukaryotes (reviewed in refs. 17, 18, 52, and 78). Components of the regulatory mechanisms of these processes have been described only for transcription-associated mutation, which involves the stringent response (amino acid starvation) (79, 80), SOS-mutagenesis in aging colonies (74, 75) and starving cells (76, 77), *phoPQ* involvement in *ebgR* mutation (81), and this report. Understanding the regulation of all of the different adaptive or stationary-phase mutation mechanisms will illuminate when, how, and whether cells adjust their mutation rates and mechanisms, thereby inducing heritable changes, and presumably increasing their options for survival.

We thank Raymond Devoret for his insight that PsiB would be the inhibitor of mutation repressed by LexA, A. Bailone for sharing her unpublished allele of *psiB*, and D. Ennis, H. Ohmori, R. Woodgate, and the *E. coli* Genetic Stock Center for providing strains. We thank R. Rivera for assistance; M. Price for media; H. J. Bull, P. J. Hastings, M.-J. Lombardo, E. Witkin, and two anonymous reviewers for comments on the manuscript; and S. Gottesman for helpful discussion. This work was supported by a Department of Defense Breast Cancer Research Graduate Fellowship (to G.J.M.), an Alberta Heritage Foundation for Medical Research graduate studentship (to R.S.H.), and National Institutes of Health Grants R01 GM53158 and R01 AI43917.

1. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (ASM Press, Washington, DC).
2. Wagner, J., Gruz, P., Kim, S. R., Yamada, M., Matsui, K., Fuchs, R. P. & Nohmi, T. (1999) *Mol. Cell* **4,** 281–286.
3. Tang, M., Shen, X., Frank, E. G., O'Donnell, M., Woodgate, R. & Goodman, M. F. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 8919–8924.
4. Iwasaki, H., Nakata, A., Walker, G. C. & Shinagawa, H. (1990) *J. Bacteriol.* **172,** 6268–6273.
5. Bonner, C. A., Hays, S., McEntee, K. & Goodman, M. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7663–7667.
6. Walker, G. C (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Curtiss R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umbarger, H. E. (ASM Press, Washington, DC), pp. 1400–1416.
7. Sassanfar, M. & Roberts, J. W. (1990) *J. Mol. Biol.* **212,** 79–96.
8. Higashitani, N., Higashitani, A., Roth, A. & Horiuchi, K. (1992) *J. Bacteriol.* **174,** 1612–1618.
9. Anderson, D. G. & Kowalczykowski, S. C. (1998) *Cell* **95,** 975–979.
10. Lin, L. L. & Little, J. W. (1988) *J. Bacteriol.* **170,** 2163–2173.
11. Fijalkowska, I. J., Dunn, R. L. & Schaaper, R. M. (1997) *J. Bacteriol.* **179,** 7435–7445.
12. Radman, M. (1975) *Basic Life Sci.* **5A,** 355–367.
13. Brotcorne-Lannoye, A. & Maenhaut-Michel, G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3904–3908.
14. Drake, J. W. (1991) *Annu. Rev. Genet.* **25,** 125–146.
15. Foster, P. L. (1993) *Ann. Rev. Microbiol.* **47,** 467–504.
16. Hall, B. G. (1993) *Curr. Opin. Genet. Dev.* **2,** 943–946.
17. Lombardo, M.-J., Harris, R. S. & Rosenberg, S. M. (1999) in *Plant Responses to Environmental Stresses: From Phytohormones to Genome Reorganization*, ed. Lerner, H. R (Dekker, New York), pp. 71–90.
18. Lombardo, M.-J. & Rosenberg, S. M. (1999) *J. Genet.* **78,** 13–21.
19. Rosenberg, S. M. (1997) *Curr. Opin. Genet. Dev.* **7,** 829–834.
20. Strauss, B. S. (1998) *Genetics* **148,** 1619–1626.
21. Finch, C. E. & Goodman, M. F. (1997) *Trends Neurosci.* **20,** 501–507.
22. Harris, R. S., Longerich, S. & Rosenberg, S. M. (1994) *Science* **264,** 258–260.
23. Cairns, J., Overbaugh, J. & Miller, S. (1988) *Nature (London)* **335,** 142–145.
24. Cairns, J. & Foster, P. L. (1991) *Genetics* **128,** 695–701.
25. McKenzie, G. J., Lombardo, M.-J. & Rosenberg, S. M. (1998) *Genetics* **149,** 1163–1165.
26. Foster, P. L., Trimarchi, J. M. & Maurer, R. A. (1996) *Genetics* **142,** 25–37.
27. Harris, R. S., Ross, K. J. & Rosenberg, S. M. (1996) *Genetics* **142,** 681–691.
28. Torkelson, J., Harris, R. S., Lombardo, M.-J., Nagendran, J., Thulin, C. & Rosenberg, S. M. (1997) *EMBO J.* **16,** 3303–3311.
29. Rosche, W. A. & Foster, P. L. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 6862–6867.
30. Godoy, V. G., Gizatullin, F. S. & Fox, M. S. (2000) *Genetics* **154,** 49–59.
31. Harris, R. S., Feng, G., Ross, K. J., Sidhu, R., Thulin, C., Longerich, S., Szigety, S. K., Winkler, M. E. & Rosenberg, S. M. (1997) *Genes Dev.* **11,** 2426–2437.
32. Rosenberg, S. M., Longerich, S., Gee, P. & Harris, R. S. (1994) *Science* **265,** 405–407.
33. Foster, P. L. & Trimarchi, J. M. (1994) *Science* **265,** 407–409.
34. Longerich, S., Galloway, A. M., Harris, R. S., Wong, C. & Rosenberg, S. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 12017–12020.
35. Miller, J. H. (1992) *A Short Course in Bacterial Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
36. Volkert, M. R., Margossian, L. J. & Clark, A. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1786–1790.
37. Little, J. W., Edmiston, S. H., Pacelli, L. Z. & Mount, D. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3225–3229.
38. Mount, D. W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 300–304.
39. Blanar, M. A., Sandler, S. J., Armengod, M. E., Ream, L. W. & Clark, A. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4622–4626.
40. Yasuda, T., Morimatsu, K., Horii, T., Nagata, T. & Ohmori, H. (1998) *EMBO J.* **17,** 3207–3216.
41. Woodgate, R. (1992) *Mutat. Res.* **281,** 221–225.
42. Mount, D. W., Low, K. B. & Edmiston, S. J. (1972) *J. Bacteriol.* **112,** 886–893.
43. Lin, L. L. & Little, J. W. (1989) *J. Mol. Biol.* **210,** 439–452.
44. Markham, B. E., Little, J. W. & Mount, D. W. (1981) *Nucleic Acids Res.* **9,** 4149–4161.
45. Horii, T., Ogawa, T. & Ogawa, H. (1981) *Cell* **23,** 689–697.
46. Harris, R. S. (1997) Ph.D. Thesis (University of Alberta, Edmonton, Alberta, Canada).
47. Clark, A. J. & Sandler, S. J. (1994) *Crit. Rev. Microbiol.* **20,** 125–142.
48. Webb, B. L., Cox, M. M. & Inman, R. B. (1999) *J. Biol. Chem.* **274,** 15367–15374.
49. McPartland, A., Green, L. & Echols, H. (1980) *Cell* **20,** 731–737.
50. Mukherjee, A., Cao, C. & Lutkenhaus, J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 2885–2890.
51. Bagdasarian, M., Bailone, A., Angulo, J. F., Scholz, P. & Devoret, R. (1992) *Mol. Microbiol.* **6,** 885–893.
52. Rosenberg, S. M., Thulin, C. & Harris, R. S. (1998) *Genetics* **148,** 1559–1566.
53. Roca, A. I. & Cox, M. M. (1997) *Prog. Nucleic Acids Res. Mol. Biol.* **56,** 129–223.
54. Myers, R. S. & Stahl, F. W. (1994) *Annu. Rev. Genet.* **28,** 49–70.
55. Rosenberg, S. M. & Motamedi, M. R. (1999) in *Embryonic Encyclopedia of Life Sciences* (Nature Publishing Group, London).
56. Galitski, T. & Roth, J. R. (1995) *Science* **268,** 421–423.
57. Foster, P. L. & Trimarchi, J. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5487–5490.
58. Lombardo, M. J., Torkelson, J., Bull, H. J., McKenzie, G. J. & Rosenberg, S. M. (1999) *Ann. N.Y. Acad. Sci.* **870,** 275–289.
59. Bull, H. J., McKenzie, G. J., Hastings, P. J. & Rosenberg, S. M. (2000) *Genetics* **154,** 1427–1437.
60. Motamedi, M. R., Szigety, S. K. & Rosenberg, S. M. (1999) *Genes Dev.* **13,** 2889–2903.
61. Michel, B., Ehrlich, S. D. & Uzest, M. (1997) *EMBO J.* **16,** 430–438.
62. Rosenberg, S. M., Harris, R. S., Longerich, S. & Galloway, A. M. (1996) *Mutat. Res.* **350,** 69–76.
63. Kuzminov, A. (1995) *Mol. Microbiol.* **16,** 373–384.
64. Rosenberg, S. M., Harris, R. S. & Torkelson, J. (1995) *Mol. Microbiol.* **18,** 185–189.
65. Bridges, B. A. (1997) *Nature (London)* **387,** 557–558.
66. Harris, R. S., Feng, G., Ross, K. J., Sidhu, R., Thulin, C., Longerich, S., Szigety, S. K., Hastings, P. J., Winkler, M. E. & Rosenberg, S. M. (1999) *Mutat. Res.* **437,** 51–60.
67. Foster, P. L., Gudmundsson, G., Trimarchi, J. M., Cai, H. & Goodman, M. F. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7951–7955.
68. Harris, R. S., Bull, H. J. & Rosenberg, S. M. (1997) *Mutat. Res.* **375,** 19–24.
69. Matic, I., Rayssiguier, C. & Radman, M. (1995) *Cell* **80,** 507–515.
70. Trusca, D., Scott, S., Thompson, C. & Bramhill, D. (1998) *J. Bacteriol.* **180,** 3946–3953.
71. Kenyon, C. J. & Walker, G. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2819–2823.
72. Kim, S. R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., Nohmi, T. & Ohmori, H. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13792–13797.
73. Tomer, G., Reuven, N. B. & Livneh, Z. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 14106–14111.
74. Taddei, F., Matic, I. & Radman, M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11736–11740.
75. Taddei, F., Halliday, J. A., Matic, I. & Radman, M. (1997) *Mol. Gen. Genet.* **256,** 277–281.
76. Zhang, Q. M., Ishikawa, N., Nakahara, T. & Yonei, S. (1998) *Nucleic Acids Res.* **26,** 4669–4675.
77. Timms, A. R., Muriel, W. & Bridges, B. A. (1999) *Mutat. Res.* **435,** 77–80.
78. Harris, R. S., Kong, Q. & Maizels, N. (1999) *Mutat. Res.* **436,** 157–178.
79. Wright, B. E., Longacre, A. & Reimers, J. M. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 5089–5094.
80. Wright, B. (1996) *Mol. Microbiol.* **19,** 213–219.
81. Hall, B. G. (1998) *J. Bacteriol.* **180,** 2862–2865.

Gregory J McKenzie
Department of Molecular and Human Genetics
Baylor College of Medicine, Rm S809
One Baylor Plaza
Houston, TX 77030-3411
ph: (713) 798-6693
fax: (713) 798-8967
greglor@ualberta.net

Education

1997-Present. Ph.D. Program, Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, USA

1997. B.Sc., First Class Honours in Cell Biotechnology, University of Alberta, Edmonton, AB, Canada

Research Experience

August 1997-Present. Graduate studies, Baylor College of Medicine, Houston, TX (with Dr. Susan Rosenberg, June 1998-present)

September 1996-July 1997. Undergraduate honours research, University of Alberta (with Dr. Susan Rosenberg)

May-September 1996. Summer research, Cross Cancer Institute, Molecular Oncology Program (with Dr. Paul Grundy)

May-September 1994, May-September 1995. Summer research, Lethbridge Agriculture and Agri-foods Research Station (with Dr. Jill Clapperton)

Honours & Awards

2002. Harold M. Weintraub Graduate Student Award given by Fred Hutchinson Cancer Center

1999-2002. United States Army Breast Cancer Research Program Fellowship

1993. General Entrance Award, University of Alberta

1992. Alexander Rutherford Undergraduate Scholarship

1992. Knights of Columbus Undergraduate Chemistry Award

Teaching Experience

2001. Teaching Assistant, Genetics A, Graduate Introduction to Genetics, Baylor College of Medicine

1998-Present. Mentor to 7 rotation students & 2 undergraduate students, (one, P. Lee, author on two papers below)

Publications

McKenzie, GJ, M-J Lombardo, & SM Rosenberg. 1998. Recombination-dependent mutation in *Escherichia coli* occurs in stationary phase. *Genetics* 149: 1163-1165.

Lombardo, M-J, J Torkelson, HJ Bull, GJ McKenzie, & SM Rosenberg. 1999. Mechanisms of genome-wide hypermutation in stationary-phase. *Annals NY Acad Sci* 870: 275-289.

Barry, ME, D Pinto-Gonzalez, FM Orson, GJ McKenzie, GR Petry, & MA Barry. 1999. Role of endogenous endonucleases and tissue site in transfection and CpG-mediated immune activation after naked DNA injection. *Hum Gene Ther* 10: 2461-2480.

Bull, HJ, GJ McKenzie, PJ Hastings, & SM Rosenberg. 2000. Evidence that stationary-phase hypermutation in the *Escherichia coli* chromosome is promoted by recombination. *Genetics* 154: 1427-1437.

McKenzie, GJ, RS Harris, PL Lee, & SM Rosenberg. 2000. The SOS response regulates adaptive mutation. *Proc Natl Acad Sci USA.* 97: 6646-6651.

Bull, HJ, GJ McKenzie, PJ Hastings, & SM Rosenberg. 2000. The contribution of transiently hypermutable cells to mutation in stationary phase. *Genetics* 156: 925-926.

McKenzie, GJ, PL Lee, M-J Lombardo, PJ Hastings & SM Rosenberg. 2001. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol Cell* 7: 571-579.

McKenzie, GJ & SM Rosenberg. 2001. Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens. *Curr Opin Microbiol* 4: 586-594.

McKenzie, GJ, PL Lee & SM Rosenberg. 2002. The SOS-regulated *dinB* operon and spontaneous mutation in *E. coli.* in preparation.

Meetings attended

Platform presentations:

2000. Lost Pines Molecular Biology Conference. Lost Pines, Texas. Biological function of *E. coli* DinB/DNA polymerase IV in adaptive mutation.

2000. Molecular Genetics of Bacteria and Phages Meeting. Cold Spring Harbor Laboratory. Biological function of *E. coli* DinB/DNA polymerase IV in adaptive mutation.

1999. Molecular Genetics of Bacteria and Phages Meeting. University of Wisconsin, Madison. The SOS response in stationary-phase mutation.

Poster presentations:

2001. Molecular Genetics of Bacteria and Phages Meeting. University of Wisconsin, Madison. Inducible, mutagenic DNA polymerase IV (DinB) in recombination-dependent adaptive mutation.

2001. Genetic Recombination and Chromosome Rearrangements (FASEB Summer Conference). Snowmass, CO. Inducible, mutagenic DNA polymerase IV (DinB) in recombination-dependent adaptive mutation.

1998. American Society for Microbiology Texas Branch Meeting. Houston, TX. Recombination-dependent mutation occurs in stationary phase.

References

Susan M Rosenberg, Ph.D.
Professor
Department of Molecular & Human Genetics
Baylor College of Medicine
One Baylor Plaza, Room S809A
Houston, Texas 77030-3411
smr@bcm.tmc.edu
ph: (713) 798-6924
fax: (713) 798-8967

Michael A Barry, Ph.D.
Assistant Professor
Department of Molecular & Human Genetics
Baylor College of Medicine
One Baylor Plaza, Room N1020
Houston, Texas 77030-3411
mab@bcm.tmc.edu
ph: (713) 798-5959
fax: (713) 798-1481

Gad Shaulsky, Ph.D.
Assistant Professor
Department of Molecular & Human Genetics
Baylor College of Medicine
One Baylor Plaza, Room S430
Houston, Texas 77030-3411
gadi@bcm.tmc.edu
ph: (713) 798-8082
fax: (713) 798-6521

Phil J Hastings, Ph.D.
Associate Professor
Department of Molecular & Human Genetics
Baylor College of Medicine
One Baylor Plaza, Room T809B
Houston, Texas 77030-3411
hastings@bcm.tmc.edu
ph: (713) 798-5787
fax: (713) 798-8704